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SEM Studies on Vessels in Ferns. 1. Woodsia obtusa

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ABSTRACT.—Circular to oval pores are present in primary walls (pit membranes) of end walls of tracheary elements in roots and rhizomes of *Woodsia obtusa* (Spreng.) Torr. (Dryopteridaceae). The pores are at least as abundant in rhizomes as in roots. We regard tracheary elements with end walls bearing these pores as vessel elements (such pores are absent on lateral walls). Pores occur in thin areas of the primary wall of pit membranes; thicker portions of pit membranes, both on lateral and end walls, occur in the form of striations oriented parallel to the long axis of the cell. These striations, recently demonstrated by means of scanning electron microscopy (SEM) for primary xylem of Nymphaeales and the monocotyledon *Acorus,* are likely widespread in vascular plants. Pore formation in *W. obtusa* is only moderate compared to that in Nymphaeales and *Acorus,* and may correlate with the fact that *W. obtusa* occupies habitats relatively more mesic than those in which other species of *Woodsia* grow.

The presence of vessel elements in ferns was claimed over a century ago by Russow (1872), who cited only two species, *Pteridium aquilinum* (L.) Kuhn and *Nephrodium filix-mas* Rich. (= *Dryopteris filix-mas* (L.) Schott), as having true perforations in the end walls. White (1962), who reviewed the history of claimed vessel presence in ferns, demonstrated that opinions varied widely: De Bary (1884) agreed with Russow (1872), whereas Gwynne-Vaughan (1908) thought that vessels could be found in Cyatheaceae, Gleicheniaceae, Hymenophyllaceae, Marattiaceae, Ophioglossaceae, Polypodiaceae, and Schizaeaceae. Bancroft (1911), using a variety of techniques, thought that vessels exist in ferns only in *Pteridium aquilinum,* a conclusion shared by Duerden (1940).

White (1961, 1962) presented clear evidence for the presence of vessels in roots of *Marsilea.* In addition, White (1962, 1963) suspected vessel presence in two other species of ferns, *Woodsia ilvensis* (L.) R. Br. and *Astrolepis sinuata* (Lag. ex Sw.) D.M. Benham & Windham (= *Notholaena sinuata (*Lag. ex Sw.) Kaulf.), although he did not demonstrate perforations. White's (1962, 1963) reason for suspecting vessels in *Astrolepis sinuata* and *Woodsia ilvensis* was marked differentiation (as indicated by distribution of pits and therefore secondary wall) between the lateral walls and the end walls of tracheary elements. In addition, moderate differences between pits of lateral walls and pits (or perforations) of end walls of tracheary elements were claimed by White (1962, p. 75) for 10 other species of *Woodsia,* and one hybrid *Woodsia.* The species we have elected to study here, *W. obtusa* (Spreng.) Torr., was cited by White (1962) as having "slight" differentiation of end walls as compared to lateral

walls of tracheary elements in roots, but he reported no differentiation between end walls and lateral walls in rhizomes of this species.

White's work was done without the benefit of scanning electron microscopy SEM. This instrument is ideally suited to examination of walls of tracheary elements and resolving pores of various sizes in the walls. Pit membrane remnants, some nearly intact, in perforation plates of primitive woody dicotyledons were shown in this way by Carlquist (1992), and the technique was extended to primary xylem of Nymphaeales, in which presence of porosities in end walls permitted designation of the tracheary elements as vessel elements, in *Barclaya* (Schneider and Carlquist, 1995a), *Victoria* (Schneider and Carlquist, 1995b), *Nuphar* and *Nymphaea* (Schneider et al., 1995) of the Nymphaeaceae, and *Cabomba* (Schneider and Carlquist, 1996a) and *Brasenia* (Schneider and Carlquist, 1996b) of the Cabombaceae. We are extending this method to monocotyledons, in which we have demonstrated porose pit membranes in end walls of tracheary elements in *Acorus,* a genus of Acoraceae formerly placed in Araceae (Carlquist and Schneider, 1997). We are also engaged in SEM studies of tracheary elements of a wide range of ferns.

Our data have potential use in showing the number of times vessels have originated in these groups; the presence of distinctive types of vessel elements, as in *Cabomba* (Schneider and Carlquist, 1996a) and *Brasenia* (Schneider and Carlquist, 1996b); and the alteration of vessel structure with relation to the aquatic habitat (Schneider and Carlquist, 1996b).

In monocotyledons, presence of vessels and their degree of specialization seem clearly related to ecology (Carlquist, 1975, p. 115). The presence of vessels in *Marsilea* seems related to seasonal drying and refilling of ponds in which that genus occurs. *Pteridium,* which has vessels throughout the plant body, occurs in sites that are dry or even frozen for prolonged periods, and it also has extraordinarily high "internal diffusive resistance" (a measure of combined resistance to transpiration from stomata, cuticle, and boundary layer), according to Gates (1968, p. 236), and occurs in localities where freezing of water in xylem in winter is very likely.

We believe that the presence of vessels in *Woodsia* may also be closely keyed to ecology. As a genus, *Woodsia* characteristically occurs in boreal latitudes and altitudes, and is most often found on outcroppings where winter freezing and summer drought may be pronounced (Brown, 1964). Examining the relationship between vessel presence and ecology is therefore a goal of our studies. *Woodsia obtusa* ranges from southern Maine and southern Québec to western Oklahoma and south-central Texas (Brown, 1964). Brown (1964) claims that *W. obtusa* is "more mesic" than its congeners, but that it is "also much more tolerant of high temperatures than other species of the genus." However, Brown's claim may be based on a generalization about elevated summer humidity in the range of *W. obtusa*. In fact, *W. obtusa* habitats may not differ much in this respect from habitats of other *Woodsia* species. *Woodsia obtusa* can characteristically be found in places such as glades and bluffs that become hot and therefore dry in the summer.

Materials and Methods

The following collection provided material for our study of *W. obtusa* subsp. *obtusa*: U.S.A., Missouri, Iron Co., along roadcut, State Highway 21, W side of road across from Royal Gorge Natural Area, plants scattered in open crevices of rhyolite cliff, with *Dryopteris, Arabis, Solidago,* and grasses, 310 m, 37° 32' 30"N, 90° 41' 10"W, 5 Oct 1995, *Yatskievych & Taylor* s.n. (SBBG).

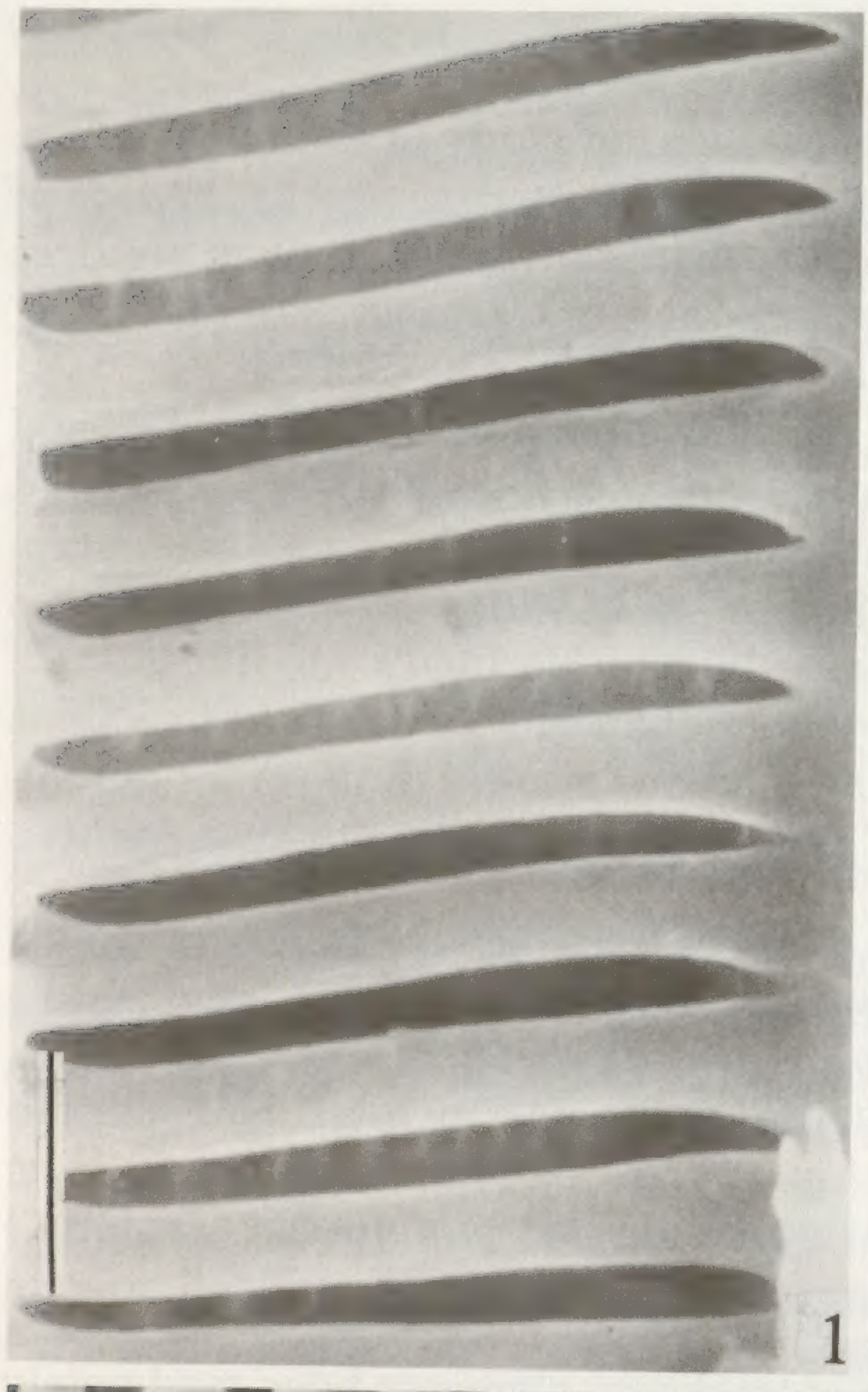
Entire plants were preserved in 50% aqueous ethanol. Portions of roots and rhizomes from these plants were infiltrated by means of the tertiary butyl series of Johansen (1940), embedded in paraffin, and sectioned on a rotary microtome. Some of the sections were mounted on glass slides and stained with a safranin-fast green combination corresponding to Northen's modification of Foster's tannic acid-ferric chloride method (Johansen, 1940). Other sections were mounted on aluminum stubs, using methods similar to those for mounting sections on glass slides, and were cleansed of paraffin and then sputter-coated for examination with SEM.

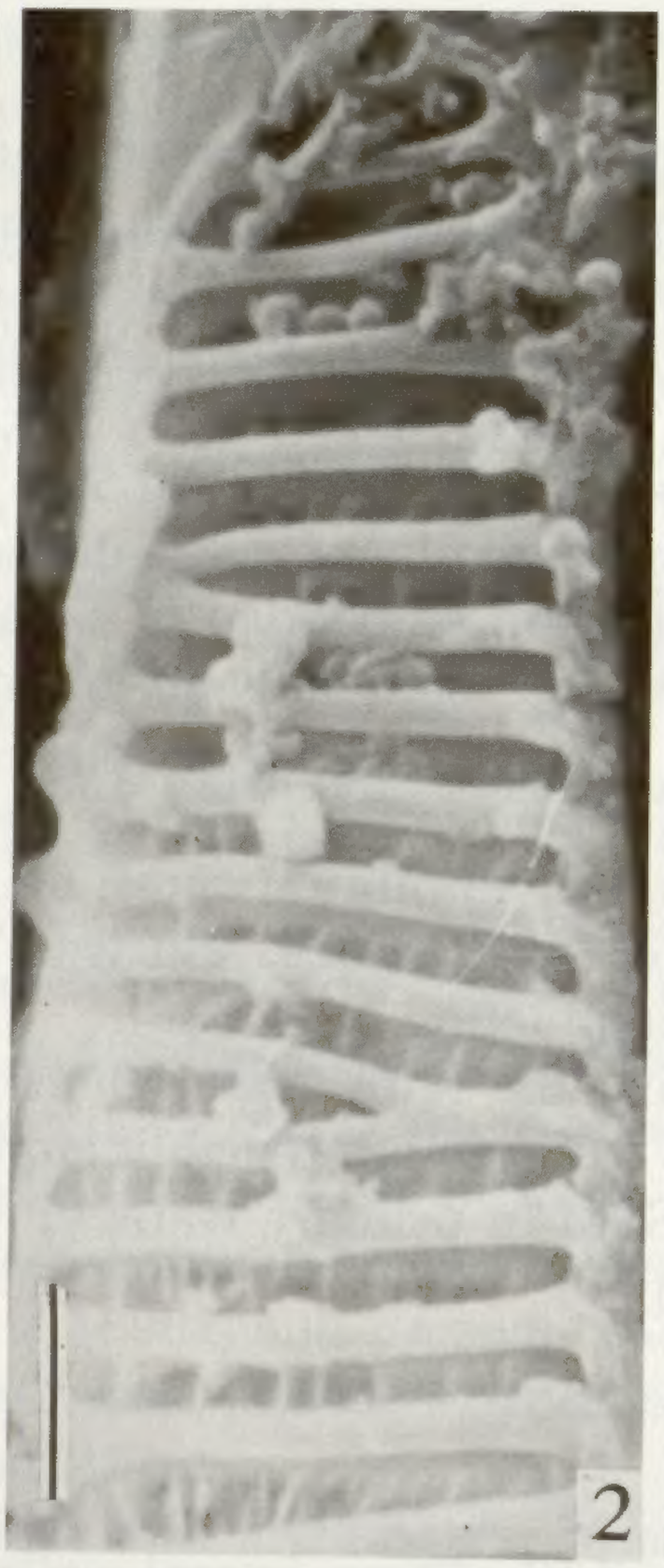
We are using the term vessel element for any tracheary element in which the presence of pores appreciably larger than those of plasmodesmata can be found with SEM in primary walls of end walls of tracheary elements, but in which such pores are absent on lateral walls.

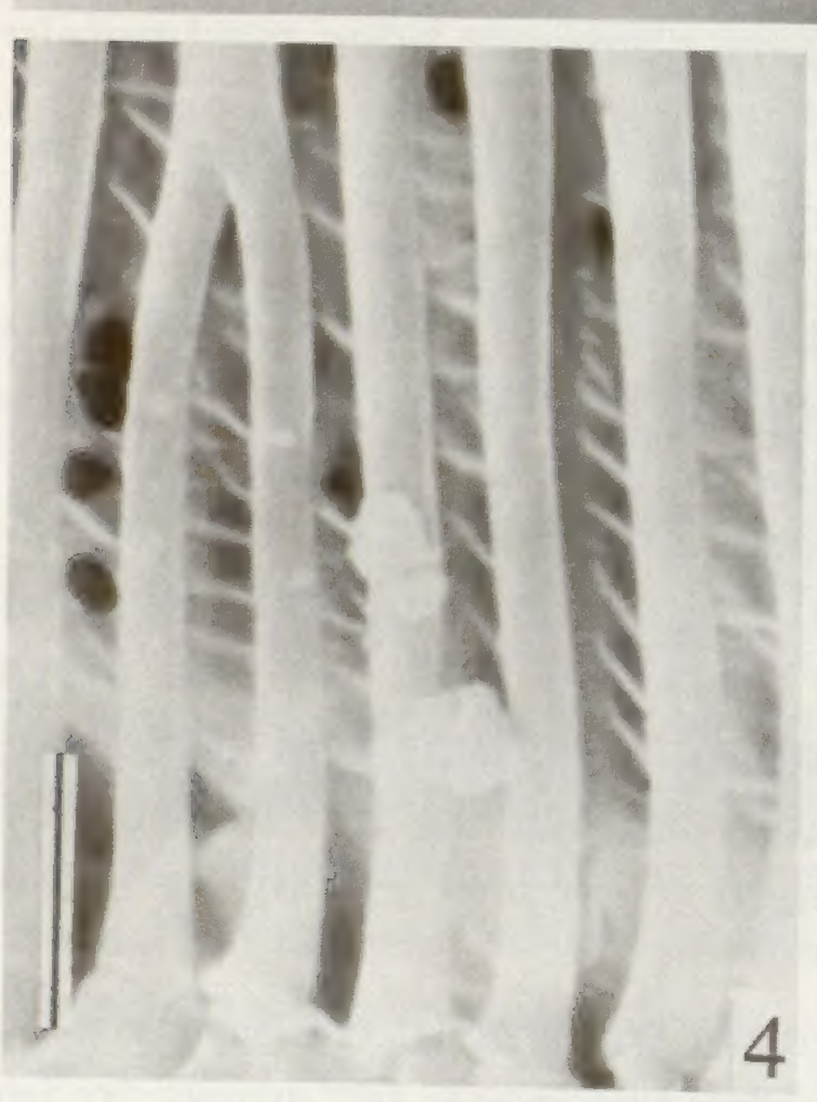
Results

In the root of *W. obtusa,* lateral walls of tracheary elements have moderately narrow pits (Fig. 1). Faint vertical striations that indicate thicker places in the pit membranes of lateral walls can be found. In the end walls of tracheary elements or roots (Figs. 2–6), pit areas are relatively wide. In the portion of an end wall shown in Fig. 2, pores are relatively few: A line of pores can be seen in each of two pit areas, the fourth and fifth from the top end of the end wall. Striations are pronounced in the pit membranes of Fig. 2, especially in the bottom two-thirds of the photograph. Larger pores are evident in the photograph of Fig. 3, which represents a longitudinal strip of an end wall. Figures 2 and 3 represent extremes in degree of pore presence; Figs. 4–6 are more typical. The end walls of Figs. 4–6 show abundant striations that represent thickenings in the primary walls. Pores of various sizes occur in the primary walls: larger in Fig. 4, smaller in Fig. 5 (Figs. 5 and 6 are portions of the same end wall).

In the rhizome of *W. obtusa* (Figs. 7, 8), pores are present in the end walls, as are the thickenings termed striations here. The tendency for pores to be circular or oval in outline is clearly shown in Figs. 7 and 8. Also, the coalescence of smaller pores close to each other into larger pores, perhaps because of progressive wall lysis that begins at particular points on the primary wall, is evident. There is no doubt that pores are present on end walls of tracheary elements of *W. obtusa.* The pores are somewhat more evident in the rhizomes than in the roots in the material we examined, but our sampling was not extensive.







FIGS. 1–6. SEM photographs of tracheary elements from roots of *Woodsia obtusa*. 1) Lateral wall pitting; faint light streaks in pits are striations (thickenings). 2) Tip of end wall, with only a few small pores in pit membranes (fourth and fifth pits from top); striations conspicuous at bottom of photograph. 3) Strip from end wall, showing pores. 4) End wall with striations and relatively large pores. 5, 6) Two portions from the same end wall. 5) Portion with small pores (above) and striations in pit membranes. 6) Portion with two coalescent pores (center, above). Scale bars = 5μm.

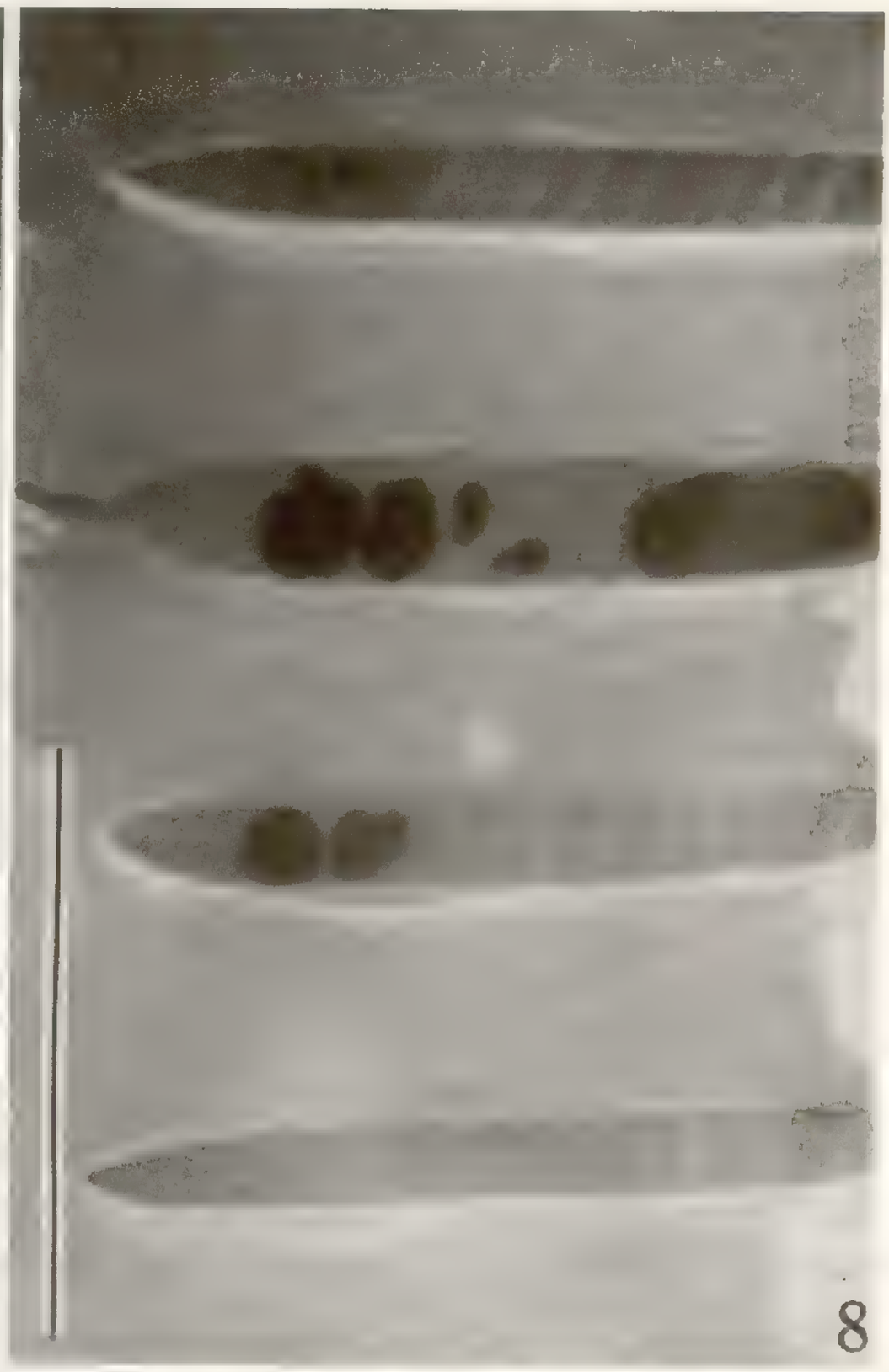


FIG. 7–8. SEM photographs of end wall portions from tracheary elements of rhizome of *Woodsia obtusa*. 7) Portion of end wall showing circular pores and striations; pits that are entirely clear of pit membranes may represent removal of membranes by sectioning. 8) Porose pit membranes, showing various degrees of coalescence; striations are also present. Scale bars = 5 m.

CONCLUSIONS

Relatively large pores are present in end walls of tracheary elements of both roots and rhizomes of *W. obtusa*. We believe these tracheary elements should therefore be termed vessel elements, although one may wish to describe the degree of presence of pit membranes in these end walls when using that term. Although White (1962) found slight differentiation between lateral and end walls of roots with respect to width of pits and bars in *W. obtusa*, he apparently found no such differentiation of rhizomes of *W. obtusa*. In our future studies of vessel presence in ferns, we will be searching for presence of vessels in ferns where there is little differentiation between end walls and lateral walls on the basis of light microscopy, as well as those species in which end walls are markedly different from lateral walls as seen with the light microscope. Interestingly, in two species of *Acorus*, a monocotyledon, we found vessels in both roots and rhizomes, although Cheadle (1942), using light microscopy, reported vessels in roots only, We also found vessels in both roots and stems of two genera of aquatic dicotyledons that have only primary xylem, *Brasenia* (Schneider and Carlquist, 1996b) and *Nelumbo* (Schneider and Carlquist,

1996c); no vessels had hitherto been reported in *Brasenia,* and vessels had been reported only for roots in *Nelumbo.*

White (1963) gave figures for pits and bars of end walls of tracheary elements in various families of ferns. He organized his figures in terms of root, rhizome, and petiole. In a table prepared from averages of end wall pit diameters for the ferns White considered (Carlquist, 1975, p. 40), the headings of two columns were by error reversed. The table should be corrected so that the pit diameters read: roots, 2.03 μm; rhizomes, 1.49 μm; petioles, 1.67 μm. The wider pits in end walls of root tracheary elements might be considered prefigurations of vessel origin in roots of ferns, at least in the sense that greater pit membrane area offers greater potential conductivity, just as a porose pit membrane offers more potential conductivity than a pit membrane without pores. If this reasoning is correct, the wider pits of end walls or root tracheary elements in ferns would be in agreement with White's (1962) generalization, which is admittedly based on limited data, that vessels tend to originate in roots in ferns and progress into stems and then petioles. This sequence is the same as that hypothesized for monocotyledons by Cheadle (1942).

The presence of lateral or adventitious roots in both ferns and monocotyledons, a condition related to lack of secondary growth in vascular bundles, may be related to this organographic sequence. Lateral or adventitious roots in these two groups of vascular plants are relatively ephemeral compared to the stems that bear them, and the lack of secondary growth in ferns and monocotyledons is correlated with this behavior: new roots must originate as older stem portions senesce. In ferns and monocotyledons, therefore, tracheary elements of roots are matured well after tracheary elements in the stem portions that bear those roots have matured: perforation plates (as recognizable with the light microscope) interconnecting stem and root tracheary elements cannot be differentiated in such a situation. Lysis of pores in pits interconnecting tracheary elements of roots with those of stems is conceivable once the root tracheary elements mature in contact with the stem tracheary elements. In dicotyledons, vessels are believed to have originated simultaneously in secondary xylem of stems and roots of particular species (Bailey, 1944). The presence of taproots intercontinuous with stems in dicotyledons, with the consequent intercontinuity of cambia and secondary xylem of stems and roots, correlates with the differential sites and sequences of vessel origins in ferns, monocotyledons, and dicotyledons, respectively. However, our knowledge of primary xylem of all three groups is very limited, because so few SEM studies have been done, and new patterns may emerge when such studies have been performed.

We note with interest the presence of thickenings that we term striations in the primary walls of pit areas both end walls and lateral walls of tracheary elements of *W. obtusa*. We believe the report of these structures in primary xylem of *Euryale* and *Victoria* (Schneider and Carlquist, 1995b) to be the first for dicotyledons, and the report of striations in *Acorus* vessels (Carlquist and Schneider, 1997) to be the first for monocotyledons. Our report of striations in *Woodsia* tracheary elements is evidently the first for ferns. The fact that stri-

ations can be found with SEM in such distantly related groups suggests that striations in primary walls of primary xylem are likely to be found much more widely. Although we do not propose a function for these structures, we note that pores in vessel elements of primary xylem, where present, occur between the striations, if striations are present. The origin of pores thus occurs in the thinner portions of the primary wall.

The pores in end walls of tracheary elements of *W. obtusa* are not large, abundant, or frequently coalescent compared to those of other vascular plants (notably those of woody dicotyledons; Carlquist, 1992). *Woodsia obtusa* represents a minimal to moderate presence of pores in end walls of tracheary elements. In future studies, we will focus on comparison of degree of pore presence and size of pores in primary walls of end walls of tracheary elements of other species of *Woodsia,* as well as in tracheary elements of ferns other than *Woodsia.* Correlation of ecology with various degrees of pore presence and perforation plate morphology will be a prime goal of our studies.

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Two *Gymnocarpium* hybrids new to the Iowa Pteridophyte flora

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ABSTRACT.—A survey of over 100 algific talus slope sites in northeastern Iowa for individuals of the genus *Gymnocarpium* revealed the presence of two additional taxa in the Iowa Pteridophyte flora. *Gymnocarpium* ×*brittonianum* (Sarvela) Pryer & Haufler was located at two adjacent sites in Clayton County, where it is disjunct approximately 300 km from the nearest reported stations in northernmost Wisconsin. *Gymnocarpium* ×*intermedium* Sarvela was located at individual sites in Clayton and Dubuque Counties. The "Driftless Area" populations for this taxon are disjunct from the northern shore of Lake Superior.

Until recently, only two taxa from the genus *Gymnocarpium* were reported from Iowa: *G. dryopteris* (L.) Newman and *G. robertianum* (Hoffm.) Newman (Peck, 1976, 1983, 1989). Recent taxonomic work by Pryer (1993) and Pryer and Haufler (1993) has demonstrated that at least five species and five hybrids occur in North America. During survey work from 1985–1991 on northeastern Iowa algific (cold producing) talus slopes (Frest, 1981), a number of specimens from *Gymnocarpium* populations were observed and collected. Examination of these materials, housed in the R.V. Drexler Herbarium (COE) at Coe College, Cedar Rapids, Iowa, revealed two additional *Gymnocarpium* taxa from the state.

Gymnocarpium ×*brittonianum* (Sarvela) Pryer & Haufler is composed of sterile, robust *Gymnocarpium* individuals that appear to be hybrids between the boreal *G. dryopteris* and the Pacific Northwest *G. disjunctum* (Rupr.) Ching (Pryer, 1993; Pryer and Haufler, 1993). The closest published records for this taxon to Iowa exist along the southern shore of Lake Superior. Large *Gymnocarpium* individuals with aborted spores were located on 7 July 1990 on an algific talus slope at Bixby State Preserve in Clayton County (Sec. 23, Lodomillo Township, *Nekola & Thomson* s.n. [COE 8499]). These specimens were examined by Kathleen Pryer in the spring of 1991 and identified as the first collection of *G.* ×*brittonianum* from Iowa. An extensive search for additional populations was made in the summer of 1991 on over 100 algific talus slope sites throughout northeastern Iowa. Only one additional clone, found on an algific slope 0.5 km east of the original colony, was located in Clayton County (Sec. 23, Lodomillo Township, *Nekola* s.n.[COE 9415]). Additionally, a third clone likely exists in the same valley, 1 km east of the original colony, but could not be definitively verified due to the early date of collection, which precluded the presence of mature spores. This taxon must be considered one

of the rarest of Iowa ferns, as it is restricted to two or three small colonies along a one km section of a single stream valley.

Gymnocarpium ×*intermedium* Sarvela is the sterile hybrid between *G. dryopteris* and *G. jessoense* (Koidz.) Koidz. ssp. *parvulum* Sarvela (Pryer, 1993; Pryer and Haufler, 1993). Whereas *G.* ×*intermedium* has been reported from Alaska to Northern Quebec, it is most abundant along the northern and western shores of Lake Superior. Although this taxon has been reported from Fillmore County, in southeastern Minnesota (K. Pryer, personal communication), no previously published reports of this taxon existed from Iowa. Two unusual *Gymnocarpium* populations with aborted spores were collected in the summer of 1990 from algific talus slope habitats in Clayton County (Sec. 26, Boardman Township, *Nekola & Thomas* s.n. [COE 9051]) and Dubuque County (Secs. 4 & 5, Taylor Township, *Nekola* s.n. [COE 8385]). These were identified by K. Pryer in the spring of 1991 as *G.* ×*intermedium*. Of these, the Dubuque County population was by far the largest, with this taxon being dominant on an open, mossy, cold northwest-facing, algific talus slope. Extensive searches in the summer of 1991 on other algific slope sites uncovered no additional populations. Subsequent examination of *Gymnocarpium* specimens from other major Iowa herbaria in 1995 by K. Pryer (pers. comm.) uncovered an additional historical (1896) collection of this taxon from Winneshiek County (*Goddard* s.n. [IA, ISC]).

Although this taxon typically occurs with both parent species in its western Great Lakes populations, neither *G. dryopteris* nor *G. jessoense* ssp. *parvulum* was located at either of the extant *G.* ×*intermedium* sites. Discovery of *G. jessoense* var. *parvulum* would be important, as it currently is not known as an extant member of the Iowa flora. Kathleen Pryer (pers. comm.) noted the existence of two herbarium specimens of *G. jessoense* var. *parvulum* collected in Iowa, portions of a July 1882 collection from Winneshiek County (*Holway* s.n. [GH]) and a September 1958 collection from Allamakee County (*Hartley 6254* [IA]). Unfortunately, the Allamake County specimen could not be located during this study and recent searches for this taxon at the site have been unsuccessful.

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Nuclear Paracrystals in the Fern *Polystichum setiferum* Gametophyte

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ABSTRACT.—Single nuclear paracrystals were observed in gametophyte cells of the fern *Polystichum setiferum*. They consist of proteins poor in basic aminoacids and have sharp-profiled, polyhedral shapes, with inner diameters up to 4.5 μm. The substructure is a fine, very close-meshed reticulum resolvable at magnifications of 40,000× or more. The hypothesis that these paracrystals are storage bodies of unused nuclear proteins is discussed.

Proteinaceous nuclear paracrystalline formations have been described in animals and plants. For example, in plants they have been found in: algal cells (Pickett-Heaps, 1967; Barton, 1967); meristematic cells of the root tip of different species of Pteridophyta (Fabbri and Menicanti, 1970); leaf cells of ferns (Weintraub et al., 1971; Fabbri and Menicanti, 1970) and an angiosperm (Perrin, 1969, 1970); photosynthesising stem cells of a fern (Menicanti, 1972); sieve elements of ferns in the Polypodiaceae (Hébant, 1969; Evert and Eichhorn, 1974) and angiosperms in the Boraginaceae (Esau and Thorsch, 1982; Thorsch and Esau, 1983); and embryo, style, and stigma cells of angiosperms (Villiers, 1968; Ciampolini *et al.,* 1983). Nuclear crystalloids have never been reported in fern gametophytes. This paper reports the electron microscopic evidence of these functionally enigmatic formations in nuclei of gametophyte cells of *Polystichum setiferum,* a species that is diffusely widespread in Europe (Tutin et al., 1993). In Italy, it is present in Alps, Pre-Alps and tyrrhenian regions (Ferrarini et al., 1986).

MATERIALS AND METHODS

Polystichum setiferum (Forssk.) T. Moore ex Woynar spores were sterilized in 1% sodium hypochlorite for few minutes. Spores were sown on Knop's medium modified according to Vaudois and Tourte (1979) in sterilized Petri plates and were exposed to "daylight" fluorescent tubes at 23°C on a 12/12 h light/dark cycle. Fragments of mature gametophytes were fixed in Karnowsky solution for 2 h at 4°C and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4°C in the dark. They were then dehydrated in ethanol and embedded in Epon 812-Araldite A/M mixture (Mollenhauer, 1964). Thin sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Sections 1–2 μm thick of samples processed for electron microscopy were stained for proteins at 45°C with 0.5% Ponceau 2R in 2% periodic acid solution at pH 1.5 (Gori, 1978).

Results and Discussion

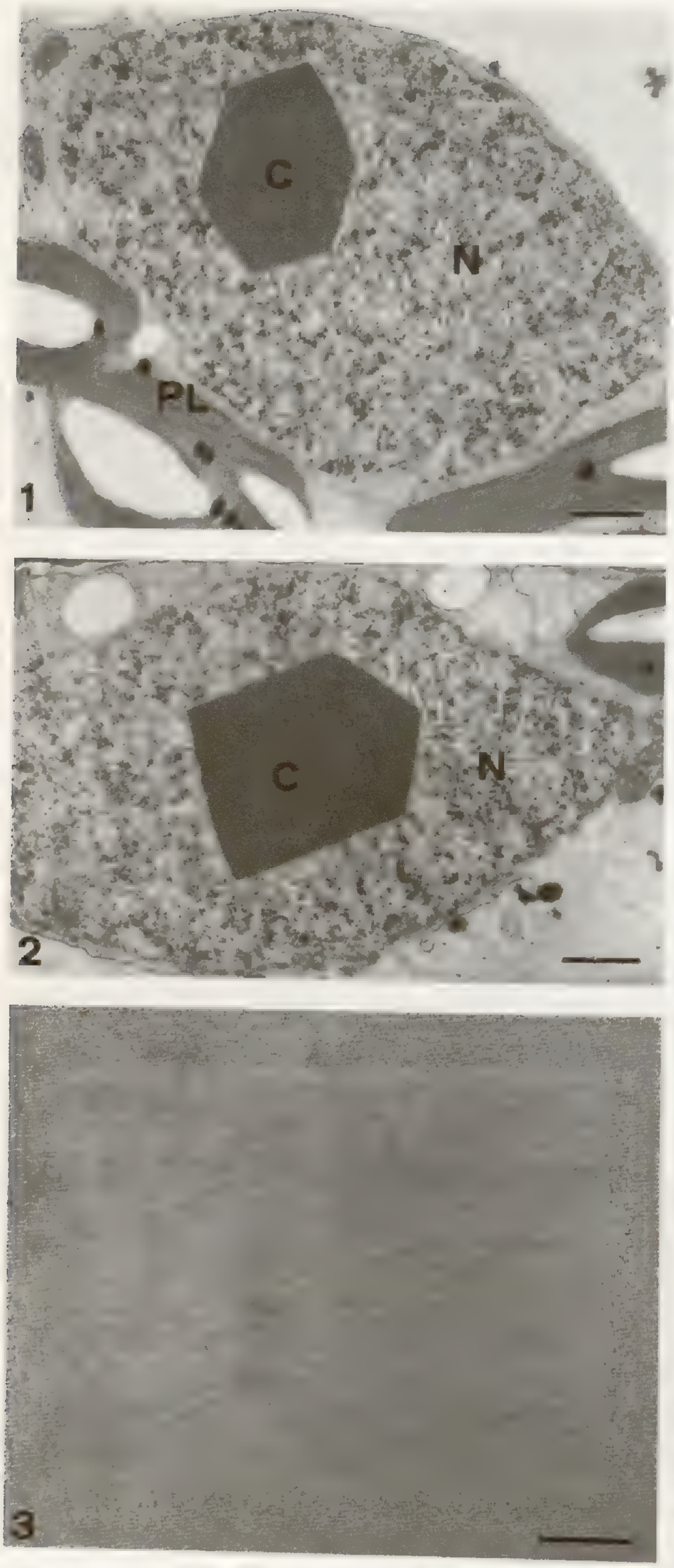
Under controlled culture conditions, four months after spores of *Polystichum setiferum* are sown, a cordate bisexual gametophyte with large tightly packed cells develops. The interphase nuclei have diffuse chromatin and are ellipsoidal, with mean longitudinal and transverse diameters of 7 and 10 μm respectively. Most of them—i.e., all except those of cells adjacent to antheridia and archegonia—contain single paracrystals (Figs. 1, 2). These paracrystals have sharp, polygonal outlines indicating regular polyhedral shapes. The diameters are up to 4.5 μm. The inner structure is a very close-meshed reticulum clearly resolvable at magnifications of 40,000× or more (Figs. 3, 4). Unlike the nucleus and nucleolus, the crystalloids are not stained by Ponceau 2R.

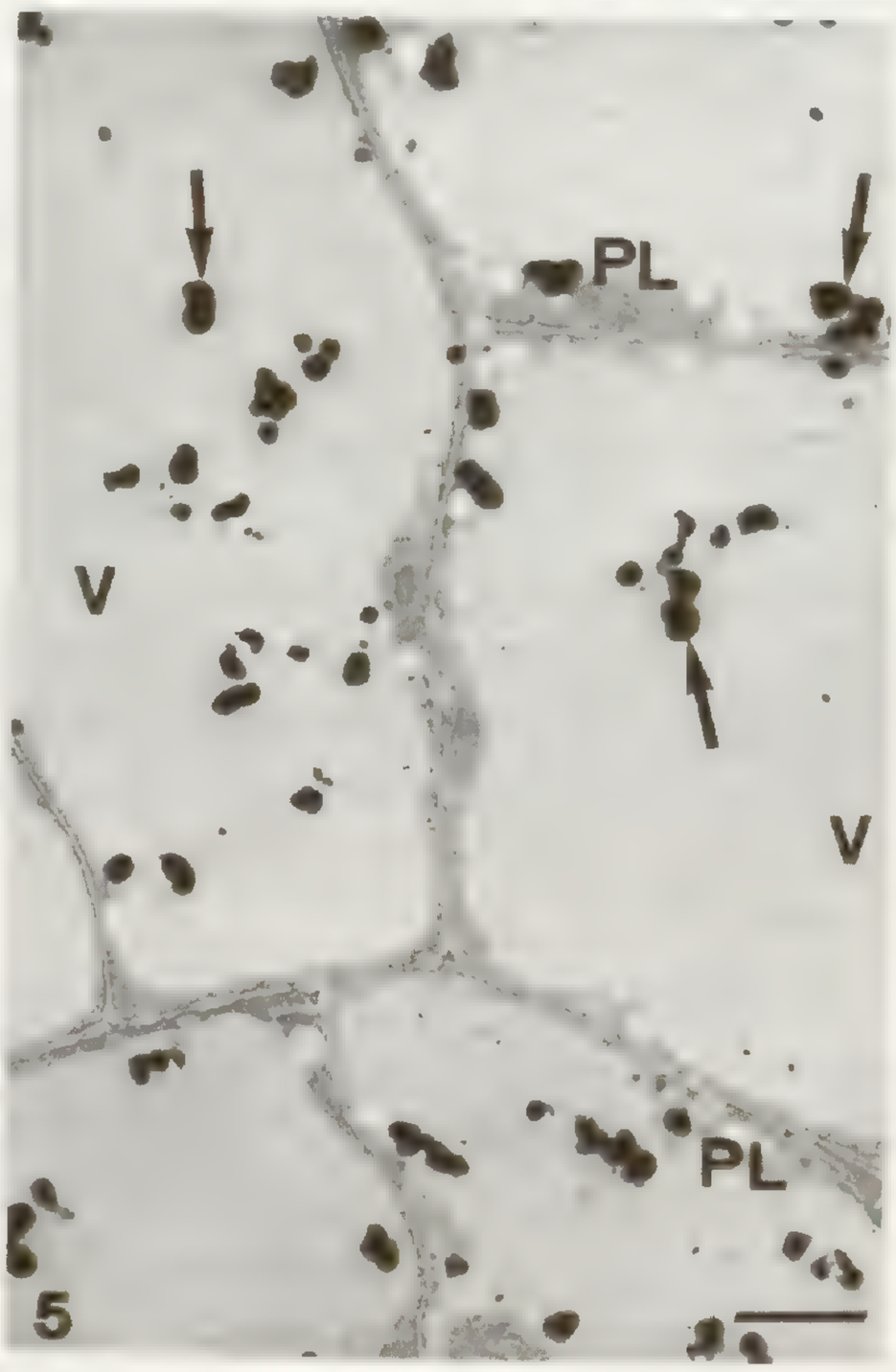
In *P. setiferum,* nuclear crystalloids have been reported previously in leaf cells (Fabbri and Menicanti, 1970). In light of the available information and the present study, both the haploid and diploid phases of the species clearly possess nuclear paracrystals. In leaf cells, the nuclear paracrystals are very rare, whereas in gametophyte cells they are abundant. Both have polygonal outlines, but those of leaf cells have a maximum diameter of 1.9 μm, less than half that of the paracrystals of gametophyte cells. Unlike those in *Pinguicola caudata, Polypodium punctatum* (Sauer, 1962, cited by Frey-Wyssling and Muhlethaler, 1965), *Asplenium nidus, Polypodium aureum,* and *Campyloneurum phyllitidis* (Wergin et al., 1970), nuclear paracrystals of *Polystichum setiferum* gametophytes are unrelated to nucleoli in size.

The sharply angular, polyhedral shape and close-meshed, reticulate substructure of the present paracrystals are two of the many previously described features of these cellular entities. Nuclear inclusions vary in shape from polygonal to more-or-less regularly spherical bodies, and the substructure has been reported as latticed, crystalline, fibrous, tubular, granular, amorphous, and even as consisting of only one or two distinct components (Fabbri and Menicanti, 1970; Wergin et al., 1970; Weintraub et al., 1971; Menicanti, 1972; Esau and Magyarosy, 1979a, b; Ciampolini et al., 1983; Thorsch and Esau, 1983).

Nuclear crystalloids are thought to be composed of proteins rich in aromatic amino acids and possibly poor in basic amino acids (Weintraub et al., 1971) or of basic proteins possibly of the histone type (Menicanti, 1972). The nuclear paracrystals of the *P. setiferum* gametophytes can be inferred to consist of proteins poor in basic amino acids, because they are not stained by Ponceau 2R (Levine, 1940).

In plants, nuclear crystalloids have been postulated to consist of reserve proteins (Frey-Wyssling and Muhlethaler, 1965). In line with this, in the leaves of Pteridophyta, these nuclear components have been assumed to "have special importance during the production of the spores" (Fabbri and Menicanti, 1970). The present findings neither support nor disprove the reserve role of the nuclear crystalloids in the *P. setiferum* gametophyte. However, cells containing these inclusions have very large vacuoles with aggregates of compact electron opaque material, few free ribosomes, no rough endoplasmic reticu-





FIGS. 1–5. Shape and structure of nuclear paracrystals of *Polystichum setiferum*. 1, 2) Mature gametophyte cells that are not adjacent to antheridia and archegonia possess single, sharp-profiled, polyhedral, nuclear crystalloids. N=nucleus; PL=plastid; scale bar=1 μm. 3, 4) Fine, very close-meshed reticulum structure of nuclear gametophyte crystalloids. Scale bar=0.2 μm. 5) Gametophyte cells with nuclear paracrystals have very large vacuoles (V) with compact electron opaque materials (arrows). The cytoplasm is a thin peripheral layer poor in organelles. Scale bar=4 μm.

lum, and few small mitochondria (Fig. 5), thus suggesting low metabolic activity, whereas the gametophyte cells adjacent to antheridia and archegonia show signs of active metabolism and have no nuclear crystalloids (Muccifora and Gori, unpublished). Hence, the buildup of the latter may be associated both with decreased nuclear and cytoplasmic activities. Even so, the crystalloids of *P. setiferum* gametophytes may consist of stored nuclear proteins, e.g., enzymes, that are not needed for the time being and are packed where they eventually will be required.

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Effects of Chromium (VI) and Humic Substances on Selected Physiological Responses of *Azolla caroliniana*

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ABSTRACT.—The independent and interactive effects of Cr(VI) and humic substances were investigated on several physiological and biochemical responses of *Azolla caroliniana*. Cr(VI) concentrations of 1 and 2 mg l^{-1} were found to cause a reduction in growth and chlorophyll *a* and *b* concentrations of *Azolla* with and without the coexistence of humic substances. The Cr(VI) toxicity on these parameters was less pronounced in the presence of humic substances. Carotenoid concentrations were not significantly affected by Cr(VI) treatments while humic substances, in the absence of Cr(VI), influenced an increase in carotenoid concentration. Anthocyanins were increased in treatments receiving Cr(VI) and humic substances, both individually and together. Increases in anthocyanins were less pronounced in treatments receiving humic substances with Cr(VI) in comparison to treatments receiving Cr(VI) in the absence of humic substances. Cr(VI) and humic substances influenced an increase in sucrose, starch, and total nonstructural carbohydrate (TNC) accumulation. In the coexistence of Cr(VI) and humic substances, accumulation of carbohydrates was reduced in comparison to those receiving Cr(VI) in the absence of humic substances. Humic substances in the absence of Cr(VI) influenced a significant decrease in the activities of sucrose phosphate synthase (SPS) and invertase and a significant increase in the activity of sucrose synthase (SS). Cr(VI)was insignificantly influenced SPS activity at 1 mg l^{-1} and at 2 mg l^{-1}. Invertase activity was significantly increased at both Cr(VI) treatments. Humic substances and Cr(VI) interactively resulted in increased SS activity insignificantly above those treatments receiving Cr(VI) in the absence of humic substances. Phosphate synthase activity was reduced at both Cr(VI) treatments when humic substances were coexistent in comparison to treatments receiving Cr(VI) in the absence of humic substances. Invertase activities were reduced in treatments receiving Cr(VI) and humic substances together in comparison to those receiving Cr(VI) in the absence of humic substances.

Chromium (Cr) finds its way into aquatic ecosystems predominantly through industrial processes such as metallurgical operations, tanning, dyeing, electroplating, and paint manufacturing (Hartford, 1979). It has been estimated that approximately 1068 metric tons of chromium are released into the biosphere annually (Nriagu, 1988). Of the various forms of chromium, the most commonly occurring are the trivalent form, Cr(III), and the hexavalent form, Cr(VI). Of these, Cr(VI) is the most readily assimilated by biotic systems and is the most toxic form of chromium known (Schroll, 1978; Towill et al., 1978).

The effects of Cr have been studied on a relatively wide range of aquatic plants. In the angiosperm, *Myriophyllum spicatum* L. (Haloragaceae), it was shown that Cr(VI) concentrations up to 50 μ l^{-1} caused an increase in shoot

growth, whereas higher concentrations resulted in a nearly linear reduction in plant growth (Guilizzoni et al., 1984). Furthermore, Staves and Knaus (1985) showed that Cr(VI) concentrations exceeding 1.0 mg l^{-1} resulted in a decrease in growth rate of the angiosperm genera, *Lemna* and *Spirodela* (Lemnaceae). Sela et al. (1989) obtained comparable results with the fern, *Azolla filiculoides* Lam. (Azollaceae), with Cr(VI) concentrations ranging from 8 to 15 μg l^{-1} reducing growth rates. Similar results were obtained by Sarkar and Jana (1986) in that Cr(VI) concentrations of 2 and 5 mg l^{-1} resulted in a decreased growth rate as well as a reduction in chlorophyll concentration of *Azolla pinnata* R. Br. However, Cr(VI) concentrations of 0.02, 0.2, 1.1 and 2.2 mg l^{-1} were shown to influence an increase in photosynthetic rates of the angiosperm, *Nelumbo lutea* (Willd.) Pers. (Nelumbonaceae) after 48 hours incubation at a pH of 5.6 (Francko et al., 1993).

In nature, chromium most likely exists in the presence of decomposed organic matter. Terrestrially derived humic acid and aqueous humic acid were shown to reduce the effects of copper toxicity to the green alga *Chlamydomonas reinhardtii* P.A. Dang. (Garvey et al., 1991). These same investigators demonstrated that aqueous fulvic acid had little or no effect on copper toxicity to this green alga. Hongve et al. (1980) reported that humic substances had no effect on the cadmium toxicity to phytoplankton photosynthesis. However, there is very limited information available on the role of naturally extracted humic substances on the influence of heavy metals on aquatic plants in general, and especially the fern genus *Azolla*. The objective of this investigation was to determine the interactive effects of two concentrations of Cr(VI) (1.0 and 2.0 mg l^{-1}) and humic substances (extracted from leaves of *Typha latifolia* L. [Typhaceae]) on *Azolla caroliniana* Willd. growth and chlorophyll, carotenoid, and anthocyanin concentration. Humic substances extracted from *Typha latifolia* were used in other physiological and biochemical investigations of planktonic and attached algae, bacteria, and vascular aquatic plants (Kim and Wetzel, 1993; Wetzel, 1993). To understand the influence of the above growth conditions on the carbohydrate status, sucrose, starch, and total nonstructural carbohydrates (TNC) were also examined.

It is generally accepted that sucrose formation in plant tissues is catalyzed by sucrose phosphate synthase (SPS), whereas the breakdown of sucrose is correlated with the activities of invertase and sucrose synthase (SS) (Hubbard et al., 1991; Miron and Schaffer, 1991). There is very little, if any, information available on sucrose metabolizing enzymes in *Azolla*, especially as affected by Cr toxicity. Therefore, sucrose metabolizing enzymes under the experimental conditions were also investigated.

Materials and Methods

Preparation of Humic Substances.—Leaves of *Typha latifolia* were chopped and placed in an aquarium filled with water and allowed to decompose for approximately one year under greenhouse conditions. Naturally occurring hu-

mic substances consist predominantly of humic acid and fulvic acid (Manahan, 1994). The decomposed organic matter was collected, filtered using Whatman filter paper (pore size 11 μm), autoclaved at 20 psi, 160 °C for 15 min., then filtered again. The filtrate was then diluted with distilled water to obtain the desirable absorbance, 0.150 at 250 nm, which was observed to be the wavelength at which peak absorbance for the humic material occurred.

PLANT MATERIAL.—Cultures of *Azolla* were grown in Hoagland solution (Hoagland and Arnon, 1938), diluted 1:40 (Sela et al., 1989) at pH 6.0. The plants were placed in the growth chamber with a 14 h photoperiod and 220 μmol m^{-2} s^{-1} photosynthetic photon flux density and day/night temperatures of 25 ± 1°C. A total of 1080 plants was randomly selected and placed in 250 ml Erlenmeyer flasks (12 flasks per treatment). Each flask contained 125 ml of Hoagland solution and received fifteen selected plants. All experimental units of each treatment received Cr(VI) concentrations of 0, 1, or 2 μg ml^{-1}, with and without humic material. Chromium was added to the medium in form of $K_2Cr_2O_7$. The pH of each sample of each treatment were adjusted to 6.0.

Azolla was grown under the above conditions for 14 days with the growth media being changed at the end of the first week. At the end of the second week of treatment, six randomly selected samples of each treatment were used for dry weight and nonstructural carbohydrates. The other six samples of each treatment were used for testing of other parameters.

GROWTH DETERMINATION.—The plants of each treatment were oven dried for 2 h at 80°C in an effort to stop enzyme action, then dried for an additional 48 h at 70°C. Dry weights were recorded and the samples stored at −20°C for subsequent determination of carbohydrates.

CHLOROPHYLL AND CAROTENOID DETERMINATION.—A sample of 0.05 mg plant material was placed in a 10 ml vial of N,N-dimethylformamide (DMF) and incubated in the dark for 36 h at 4°C in order to extract chlorophyll. Chlorophyll *a* and *b* content were determined spectrophotometrically by the method of Inskeep and Bloom (1985). Carotenoid content was determined spectrophotometrically from the N,N-DMF extraction, and total concentration was calculated using the formula of Doong et al. (1993).

ANTHOCYANIN DETERMINATION.—A sample of ground plant material, approximately 0.10 g fresh weight, was homogenized in 5 ml methanol containing 1% HCl (v/v) for 2 min on ice. The homogenate was filtered and absorbance of the extract was determined spectrophotometrically by the method of Mancinelli (1990).

CARBOHYDRATE DETERMINATION.—Carbohydrate analysis of the plant samples was conducted following a slightly modified procedure from that outlined by Chatterton et al. (1987). A portion of known weight of each sample of *Azolla* was analyzed for soluble sugars, starch, and total nonstructural carbohydrates (TNC).

To determine soluble sugar concentration, dried samples were ground to a

fine powder and extracted with 5 ml 95% ethanol at 90°C for 30 min. The samples were then centrifuged at 4,000× g for 10 min and the supernatant collected. Another 5 ml 95% ethanol was added to the precipitate and remaining soluble sugars were collected as above. The supernatants were combined and used to determine total soluble sugars following the method of Dubois et al. (1956). A 0.1 ml aliquot from each of the combined ethanol extractions samples were set aside for sucrose determination following the procedure described by Van Handel (1968).

The residue remaining after ethanol extraction was used for starch determination. The residue was resuspended in 2 ml of distilled water and then placed in the hot water bath at 90°C for 1 h to gelatinize the amylopectin. Following cooling, 5 ml of 0.2M acetate buffer (pH 4.6) containing 0.5% (w/v) porcine pancreatic alpha amylase and 2% (w/v) *Rhizopus* mold amyloglucoside were added to the residue. The samples were incubated at 25°C for 1 h and 55°C for 24 h. The following days samples were centrifuged at 10,000× g for 15 min and the supernatant was collected. Five ml of water were added to the precipitate and the samples were placed in a water bath at 60°C for 10 min and recentrifuged. The combined supernatants from the two water extracts were used for glucose determination, as for ethanol extracts to determine starch concentration. The remaining residue was hydrolyzed with 5 ml of 0.6N HCl at 80°C for 1 h. Samples were centrifuged and supernatant collected and glucose was measured as in above in order to determine the residual carbohydrate in the samples. Total nonstructural carbohydrate concentration was obtained by combining the concentration of soluble sugar, starch, and the results from the HCl hydrolysis together (Chatterton et al., 1987).

ENZYME EXTRACTION.—Approximately 0.5–1.0 g fresh weight of the plants was homogenized in 10–15 ml of extraction medium containing: 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM $MgCl_2 \cdot H_2O$, 1 mM Na Ethylenediaminetetraacetic acid (EDTA), 2 mM diethyldithio- carbamic acid (DIECA), 2.5 mM DL Dithiothreitol (DTT), 2% (w/v) insoluble Polyvinylpyrrolidone (PVP), according to the method of Hubbard et al. (1989). The extract was centrifuged for 30 min at 18,000× g. The supernatants were dialyzed for 16 h at 4°C against 25 mM Hepes-NaOH (pH 7.5).

SPS AND SS ASSAY.—A 140 µl aliquot of tissue extract was incubated at 37°C for 15 min with an equal volume of a mixture containing 15 mM UDP-glucose, 15 mM fructose 6-P, 5 mM $MgCl_2 \cdot 6H_2O$, 5 mM $Na_2MoO_4 \cdot 2H_2O$ and 50 mM Hepes-NaOH buffer (pH 7.5) for sucrose phosphate synthase assay. For sucrose synthase assays, the fructose 6-P was replaced by fructose. Reactions were terminated by the addition of 70 µl of 1N NaOH. Sucrose concentrations were determined following the method of Van Handel (1968) and compared to appropriate sucrose standards.

INVERTASE ACTIVITY.—Invertase activity was determined by measuring reducing sugars formed from sucrose hydrolysis by the Somogyi method as modified by Nelson (1944). Invertase was assayed at 30°C in digests containing 0.5 ml

TABLE 1. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on *Azolla caroliniana* growth. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	Plant dry weight (mg)
Control (without humic and Cr)	17.95a
Humic	15.32b
1.0 Cr with humic	12.90c
1.0 Cr without humic	12.11c
2.0 Cr with humic	11.83cd
2.0 Cr without humic	10.83d

enzyme solution and 0.5 ml acetate buffer (0.02M, pH 7) containing 5% (w/v) sucrose. The assay was performed for 20 min. One half ml of the Nelson-Somogyi copper reagent was added to terminate the reaction, and the tubes were immediately capped and heated for 30 min in a boiling water bath. After cooling, 0.5 ml of the arseno-molybdate reagent was added and followed by 3.5–4 ml of H_2O. The absorbance was read at 660 nm and compared to the appropriate concentration of glucose standard.

STATISTICAL ANALYSIS.—This experiment was repeated twice and analyzed statistically as a randomized complete design (Steel and Torrie, 1980). This design ensured that observed differences in plants' performances were due to treatments, rather than variations among blocks (replicate series conducted at different times). Mean separations for the values that showed significant F values (P = 0.05) of the ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The presence of the chromium in the growth media caused a significant reduction in *Azolla* growth (Table 1). This decrease in growth of Cr(VI)-treated plants may be due in part to a reduction in nutrient uptake. Sela et al. (1989) reported that Cr(VI) exposure influenced a decrease in the essential nutrients Ca, Mg, and K. It also was evident that the growth decreased significantly with an increase in chromium concentration. Similar results were observed by several investigators, most recently by Gaur et al. (1994).

The presence of the humic substances appeared to reduce chromium toxicity to *Azolla,* at both Cr(VI) concentrations (1 and 2 mg l^{-1}), which were shown respectively an 8.9 and 9.2% growth increase in comparison to that obtained of plants grown at the same Cr(VI) concentration in the absence of humic substances. De Filippis and Palaghy (1994) suggested that bioaccumulation of any metal is a function of its ionic level in the growth media. The ionic level of the Cr(VI) was probably reduced by binding to the humic substances, thus limiting its availability to the plant. Stackhouse and Benson (1988) found that the presence of 5.0 and 50 mg l^{-1} humic acid reduced the concentration of free chromium in the media. *Azolla* growth was significantly reduced by the

TABLE 2. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on chlorophyll *a* (Chl*a*) and chlorophyll *b* (Chl*b*) concentrations in *Azolla caroliniana*. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} fresh weight		
	Chl*a*	Chl*b*	Chl*a*:Chl*b*
Control (without humic and Cr)	9.78a	8.85a	1.11a
Humic	9.96a	9.84a	1.06a
1.0 Cr with humic	5.09b	2.89b	1.77b
1.0 Cr without humic	3.52c	2.06b	1.73b
2.0 Cr with humic	5.31b	2.86b	1.87c
2.0 Cr without humic	3.42c	2.03b	1.70b

presence of the humic substances in the absence of Cr(VI) (Table 1). This indicates that although humic substances reduced the toxicity of the Cr(VI) to *Azolla* growth, probably through chelation, they may also exhibit an inhibitory effect on *Azolla* growth, possibly through chelating essential nutrients.

The concentration of chlorophyll *a* and *b* of *Azolla* declined significantly in the presence of Cr(VI) (Table 2). This finding was in marked contrast to that reported previously for *Azolla,* in which Sarkar and Jana (1986) found an insignificant reduction in chlorophyll concentration at 2 and 5 mg l^{-1} Cr. However, Bassi et al. (1990) showed that a significant reduction in chlorophyll concentration of *Lemna minor* was obtained with Cr(VI) concentrations ranging from 0.1 to 10 μg l^{-1}. In the same investigation, a decrease in the chlorophyll concentration in *Pistia stratiotes* L. (Araceae) was shown at Cr(VI) concentrations of 1 to 20 μg l^{-1}. Cr(VI)-induced reduction in chlorophyll concentration may be due to swelling of the chloroplast or thylakoid disarrangement (Bassi et al., 1990) or an increase in chlorophyllase activity (Sen *et al.* 1987).

Humic substances once again seemed to influence a significant reduction in the toxic effects of Cr(VI). Humic substances reduced the effect of Cr(VI) to *Azolla* chlorophyll *a,* but exhibited no significant influence on Cr(VI) effect on chlorophyll *b* (Table 2). In the absence of Cr(VI), humic substances had no influence on chlorophyll *a* or chlorophyll *b.* The ratio of chlorophyll *a* to chlorophyll *b* was significantly increased in the presence of the Cr(VI) (Table 2). Furthermore, the presence of humic substances with Cr(VI) resulted in greater increase in the ratio of chlorophyll *a* to chlorophyll *b.* This result indicates that chlorophyll *b* was more susceptible than chlorophyll *a* to the presence of Cr(VI) with or without humic substances in the growth media.

Carotenoid concentrations were not affected by the presence of Cr(VI) with or without humic substances (Table 3). However, a 54.2% increase in the carotenoid concentration was obtained in plants grown in growth media containing humic substances without Cr(VI). Anthocyanin concentration increased in response to Cr(VI) exposure (Table 3), both 1 and 2 mg l^{-1} Cr(VI) resulted in a significant increase in comparison to controls. However, anthocyanin concentration was unchanged with increasing Cr(VI) concentration. Humic substances in the absence of Cr(VI) also influenced a significant in-

TABLE 3. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on concentrations of carotenoids and anthocyanins in *Azolla caroliniana*. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	µg g^{-1} fresh weight	
	Carotenoids	Anthocyanins
Control (without humic and Cr)	64.42a	5.85a
Humic	99.36b	6.10b
1.0 Cr with humic	72.78a	6.70c
1.0 Cr without humic	61.11a	7.58d
2.0 Cr with humic	74.62a	6.69c
2.0 Cr without humic	60.12a	7.25d

crease in the anthocyanin concentration. The increase in anthocyanin concentration occurred in response to the presence of humic substances in the growth media even in the absence of Cr(VI). This conclusion also was supported by Doong et al. (1993), who concluded that anthocyanins are produced by most aquatic plants in response to stress factors, such as high light intensity, high temperature, or nutritional limitations, and can be used as a stress indicator. Humic substances appeared to reduce anthocyanin concentration in those treatments receiving Cr(VI) in comparison to the same Cr(VI) concentration in the absence of humic substances.

Accumulation of total nonstructural carbohydrates increased significantly in *Azolla* grown in the presence of Cr(VI) at both concentrations (Table 4). These results indicate that Cr(VI) was probably less inhibitory to *Azolla* photosynthesis than it was to overall growth, thereby confirming the observations made by Schroll (1978) while investigating Cr toxicity to the alga *Chlorella pyrenoidosa* Chick. In the present study, the observed increase in carbohydrate accumulation by plants treated with Cr(VI) might be due to a reduction in carbohydrate utilization. Azcon-Bieto (1983) reported that lower rates of carbon assimilation and a decrease in yield were associated with carbohydrate accumulation in many plant species. A reduction in plant respiration in response to Cr(VI) exposure was reported by Bassi et al. (1990). Cr(VI) was also found to cause mitochondrial damage (Fasulo et al., 1983). Humic substances were shown to influence an insignificant increase in TNC accumulation in comparison to that of the control plants in the absence of Cr(VI), and a significant reduction in TNC accumulation in comparison to that of plants when Cr(VI) was present. The same result was obtained for starch (Table 4).

The presence of humic substances and Cr(VI), singly or combined, resulted in an increase in the sucrose concentration (Table 4). However, this increase was insignificant in the presence of humic substances with or without 1 mg l^{-1} Cr(VI). Increased Cr(VI) concentrations caused an elevation in sucrose accumulation. Treatments receiving both humic substances and Cr(VI) exhibited a slight and insignificant decrease in sucrose accumulation in comparison to that obtained of the same Cr(VI) treatment in the absence of humic substances.

TABLE 4. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on sucrose, starch, and accumulation of total nonstructural carbohydrates (TNC) in *Azolla caroliniana*. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} dry weight		
	Sucrose	Starch	TNC
Control (without humic and Cr)	15.28a	276.77a	349.45a
Humic	17.77ab	297.83a	382.45a
1.0 Cr with humic	19.71abc	376.84b	466.17b
1.0 Cr without humic	21.51bc	379.05b	480.16b
2.0 Cr with humic	22.83bc	388.12b	496.03b
2.0 Cr without humic	23.69c	392.08b	503.80b

These higher concentrations of sucrose are not indicative of a higher rate of synthesis, but may result from increased accumulation in the vacuole (Gieger et al. 1983).

The activity of SS was significantly higher in *Azolla* exposed to humic substances (Table 5). Humic substances in the presence of Cr(VI) seemed to influence a slight insignificant increase in SS activity in comparison to that obtained of the same Cr(VI) treatment in the absence of the humic. With the exception of the Cr(VI) concentration of 1 mg l^{-1} without humic, the activity of SS was significantly higher in *Azolla* grown in the presence of Cr(VI). However, there were insignificant differences in SS activity between the Cr(VI) concentrations. The increase in SS activity in *Azolla* grown in the presence of Cr(VI) with or without the association of humic substances could be used to explain the fact that starch accumulation increased with these treatments. Wang et al. (1993) reported that SS was the predominant enzyme catalyzing the first reaction of starch formation that results in increasing glucose concentrations through sucrose hydrolysis. Furthermore, Doehlert (1990) reported that increased SS activity was associated with starch synthesis.

On the other hand, invertase activity was reduced in the presence of the humic substances, with and without Cr(VI). However, this reduction in activity was insignificant at the Cr(VI) concentration of 1 mg l^{-1}. The observed reduction in invertase activity in the presence of humic substances might be due to a formation of an enzyme-humic substance complex, which might result in reducing the enzyme's activity. Wetzel (1993) reported humic substances extracted from decomposed *Typha* were shown to markedly suppress phosphatase activity of planktonic and attached algae, bacteria, and vascular aquatic plants. In the absence of the humic substances, Cr(VI) influenced a significant increase in invertase activity in *Azolla* grown at both concentrations. Wang et al. (1993) reported a constant positive relationship between SS activity, but not that of invertase, and starch content. As with invertase, humic substances also resulted in a reduced activity of SPS (Table 5). A Cr(VI) concentration of 1 mg l^{-1} influenced an insignificant increase in SPS activity. However, SPS activity of *Azolla* grown in a media containing 2 mg l^{-1} Cr(VI) with or without

TABLE 5. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on the activity of sucrose metabolizing enzymes in *Azolla caroliniana*: sucrose synthase (SS); sucrose phosphate synthase (SPS); and invertase. Invertase activity measurements represent mg glucose g^{-1} fr. wt. $min.^{-1}$, whereas SS and SPS activities measurements represent mg sucrose g^{-1} fr. wt. $min.^{-1}$. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} fresh weight $min.^{-1}$		
	SS	SPS	Invertase
Control (without humic and Cr)	0.67a	0.82a	55.37a
Humic	0.41b	0.57b	35.35b
1.0 Cr with humic	0.58c	0.79a	53.86a
1.0 Cr without humic	0.62ac	0.85a	60.72c
2.0 Cr with humic	0.57c	0.52b	48.96d
2.0 Cr without humic	0.58c	0.70ab	58.96c

humic substances was significantly reduced. This result indicated that the influence of Cr(VI) on SPS activity is related to Cr(VI) concentration. Nomura and Akazawa (1974) reported that metal ions including Co^{+2}, Fe^{+2}, $Fe,^{+3}$, Mg^{+2}, and Mn^{+2} stimulated the activity of SPS at relatively low concentrations. In this investigation, the increase in sucrose concentration in the presence of humic substances and with increased Cr(VI) concentrations was not correlated with the increase in SPS activity (Tables 4 and 5). Sucrose was found to be a potential inhibitor of SPS in several studies (Rufty and Huber, 1983; Huber, 1981; Salerno and Pontis, 1978). Similar to our findings, Huber and Israel (1982) reported a negative relationship between the activity of SPS and starch accumulation in leaf extracts of *Glycine max* L. (Fabaceae) exposed to a variety of environmental and nutritional conditions.

In conclusion, results from the present study indicate that reductions in growth and chlorophyll production and increases in anthocyanins and carbohydrate accumulation in *Azolla* were not as great in those treatments receiving humic substances in addition to Cr(VI) in comparison to the treatments that received Cr(VI) in the absence of humic substances. These results suggest that the presence of humic substances decreased the toxic effects of Cr(VI). In addition, Cr(VI) caused an increase in the activities of sucrose metabolizing enzymes, with the exception of SPS activity which was decreased at 2 mg l^{-1}. Humic substances caused an increase in SS activity in the absence of Cr(VI), but decreased SPS and invertase both with and without Cr(VI). The greatest reduction in the activities of SPS and invertase occurred when humic substances were present without Cr(VI).

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Shorter Note

Ferns at Fort Adams State Park, Rhode Island.—The occurrence of ferns at Fort Adams in Newport, Newport County, Rhode Island, parallels remarkably their occurrence at Fort Totten, Queens County, New York, as reported by Greller and Locke (Amer. Fern J. 73:6–8, 1983). Greller and Locke found *Asplenium platyneuron* (L.) Britton Sterns, & Poggenb. and *Woodsia obtusa* (Spreng.) Torr. flourishing in decomposing mortar that holds in place the blocks of granite of the fort walls. At Newport, the *Woodsia* likewise thrives in mortar of the exterior walls (Fig. 1), but not the *Asplenium,* although *Cystopteris tenuis* (Michx.) Desv. in lesser numbers occupies more sheltered spots within the fort near open portals.

The similarities increase when one realizes that the same Joseph G. Totten, who is celebrated at the Queen's County fort, engineered in 1824 the remodeling of the already outmoded Fort Adams while he was a lieutenant colonel. As the Queens County ferns have adapted to salt spray, so at Newport, where the walls are less than 100 ft from Narragansett Bay (Fig. 2). They take the spray from the prevailing northwest winter winds and nor'easters assault them frequently. Yet, hundreds of *Woodsia* plants not only survive, but grow and bear copious spores. In addition to the ferns, the yellow wall lichen (*Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale) abounds, as well as various flowering plants, from the tiny pearlwort (*Sagina procumbens* L.) to the seaside goldenrod (*Solidago sempervirens* L.).

The Fort Adams grounds, whose nearly 57 hectares (140 acres) are now a state park, rest on calcium-bearing slate. The *Asplenium platyneuron,* although not on the fort structure proper, occurs along the shore, where relentless wave action has exposed slate outcrops. It also appears on a low retaining wall, a part of the landscaping. Where blasting of rock occurred in times past, *Dryopteris marginalis* (L.) A. Gray grows along with *Selaginella rupestris* (L.) Spring, *Saxifraga virginiensis* Michx., and *Aquilegia canadensis* L. Perhaps most surprisingly of all, because of its acknowledged rarity in Rhode Island, is a colony of *Asplenium trichomanes* L. on a ledge obviously blasted to make way for a road.

At an abandoned granite quarry less than 1.5 km from Fort Adams, the writer found a stand of long beech fern, *Phegopteris connectilis* (Michx.) Watt. This grows less than 1 km from the ocean and probably not elsewhere within a 25 km radius. As recorded by this writer (Rhodora 96:102, 103, 1994), the marsh fern (*Thelypteris palustris* Schott) occupies a wall cranny along a busy street, a far cry from its usual habitat. Again, *Cystopteris tenuis* occurs on a sea cliff near Fort Adams, about 2.5 m above high tide. One can almost touch the fern and salt water at the same time. Thus, Newport, the City by the Sea, with its numerous mansions a century old or more, plays host to many fern colonies, but none more conspicuous than the *Woodsia obtusa* at Fort Adams.—Richard L. Champlin, 15 Priscilla Rd., Jamestown, RI 02835.

FIG. 1. *Woodsia obtusa* growing in the mortar of an exterioir wall at Fort Adams State Park.

FIG. 2. Exterior view of a portion of Fort Adams, showing the proximity of the exterior walls to the water of Narragansett Bay.

American Fern Journal 87(1):30–32 (1997)

Reviews

Flora Mesoamericana, Volumen 1: Psilotaceae a Salviniaceae, edited by R. C. Moran and Ramón Riba. 1995. Universidad Nacional Autónoma de México, Missouri Botanical Garden, and The Natural History Museum (London). ISBN 968-36-4700-6. Available in the U.S. from the Missouri Botanical Garden, Scientific Publications Department, P.O. Box 299, St. Louis, MO 63166. $77.50. xxii, 471 pp., clothbound.

Thirty pteridologists—principally Robbin C. Moran—contributed to this volume, which includes 1358 species placed in 136 genera. The Flora covers the area from the Mexican states of Tabasco and Chiapas and the Yucatán Peninsula southeast through the country of Panama to its border with Colombia. The Flora was ably translated into straightforward Spanish, and so will have wide readership among both Latin American botanists and those whose first language is not Spanish.

The families of ferns and fern-allies are arranged systematically, but the genera and species are each in alphabetical order. In the case of the family Pteridaceae, which is used in its broadest sense to include *Ceratopteris, Acrostichum, Neurocallis,* and *Pteris,* this causes complications in the key to the families and places related genera far apart in the text. The key to the families seems mostly workable, although I have never favored using anatomical characters (the number of vascular bundles in the stipes) in such keys, for the observations are difficult to make and destructive of herbarium specimens.

The species treatments are concise, but have sufficient detail, especially in the descriptions, to be useful. They include the accepted name and its type, synonyms only from the Flora area without types, a dozen or more lines of description, a brief but precise habitat statement, one specimen citation from each country or state of Mexico or the Yucatán Peninsula with collector, number, and herbarium of deposit, the elevational range in the Flora area, and the wider range of the taxon. The link between this volume of Flora Mesoamericana mentioned in the introduction and the TROPICOS database maintained at the Missouri Botanical Garden does not extend to specimen data at this time. However, these data will eventually be accessible on the internet.

Overall, the taxonomic treatments are well prepared, which is often difficult when working with specimens over such a wide area. The treatment of *Asplenium* breaks some new ground in suggesting groups within the genus, although it does not assign all the Mesoamerican species to these groups. I did notice a few species that might have been handled differently. *Osmunda regalis* in the New World is divisible into the northern var. *spectabilis* and southern var. *brasiliensis,* both of which occur in the Flora Mesoamericana area. Warren H. Wagner, Jr., demonstrated a few years ago that *Actinostachys germanii* is a presumably juvenile phase of *A. pennula. Loxomopsis costaricensis* differs from the South American species *L. pearcei,* to which the Mesoamerican material is attributed, in the characters elucidated nearly a century ago by

Hieronymus; the spore differences are readily seen in SEM preparations. *Solanopteris* is said to be the only myrmecophilous genus of New World ferns; however, ants are known to nest under the flattened, adherent rhizomes of the South American *Microgramma megalophylla*. The Costa Rican Atlantic slope endemic *Polypodium subviride* is placed as a synonym of *P. loriciforme,* which is endemic to central Panama. The two differ sharply in lamina shape, pinna shape, and indument.

A bibliography, glossary, and index conclude the volume. The bibliography contains a large proportion of recent taxonomic papers covering Mesoamerica and sometimes beyond. A few of the words in the glossary have definitions widely divergent from usual. Compare, for instance, the definitions of agudo and cirroso with those in B. D. Jackson's "A Glossary of Botanic Terms."

In accounting for a large and complex flora, as the Flora Mesoamericana does, some concessions have to be made to keep the published work to manageable size. In this case, one of the concessions was to not have illustrations. However, it is possible to find illustrations of most of the species in other recently published Central American fern Floras.

This is certainly one of the most important pteridophyte Floras to be published in this century. The authors and editors are to be congratulated. Everyone with an interest in the Mesoamerican ferns should have this book.—DAVID B. LELLINGER, U. S. National Herbarium MRC-166, Smithsonian Institution, Washington, DC 20560.

Scandinavian Ferns, by Benjamin Øllgaard, illustrated by Kirsten Tind. 1993. Rhodos International Science and Art Publishers. ISBN 87-7245-530-6 (paperbound), 87-7245-532-2 (hardcover). 317 pp. Available in the U.S. from Art Consulting: Scandinavia, 25777 Punto de Vista Dr., Monte Nido, Calabasas, CA 91302-2155 (tel. 818-222-1122). $89.50 (paperbound), $125.00 (hardcover).

This lavish, folio-size volume, which was first reviewed in Fiddlehead Forum 21:1 (1994), is once again available from a U.S. distributor. It covers 72 species and an additional 11 infraspecific taxa of ferns and fern allies that inhabit the Scandinavian countries of Denmark, Norway, and Sweden, the majority of which also occur in northern North America. The introduction briefly presents the basics of Scandinavian climate and geography, and explains that the low diversity and level of endemism relative to other boreal regions around the world (only one taxon is endemic to the region) is due to the harsher effects of Pleistocene glaciation in northern Europe than elsewhere. It also discusses at length the various life-history characteristics of pteridophytes and other factors that have affected taxonomic diversity, such as hybridization, polyploidy, and apogamy. These lucid explanations are a wonderful way for amateurs to learn about the processes that continue to be the topics of many taxonomic and evolutionary research papers in pteridology. The introduction concludes with a discussion of nomenclature and a key to the genera.

A glossary and bibliography occur at the end of the book. However, most of the volume consists of lengthy treatments of the taxa, including keys, descrip-

tions, and summaries of ecology and distribution. The descriptions are written in a "semitechnical" style that makes them easy to read, but preserves the scientific content. What sets this volume apart from most modern pteridofloras is the attention paid to details of distribution and autecology, as well as the amount of space given to conservation aspects, plant uses, and common names (including etymology).

Accompanying the text is a series of 103 black and white figures and 114 color plates. Artist Kirsten Tind, whose work has appeared in a number of technical books and papers, including installments of the Flora of Ecuador, Flora of Thailand, and Flora Nordica series, beautifully captures the Scandinavian pteridophytes in her color plates, several of which are habit studies including associated plants, which are identified in the legends. The black and white figures are clear and useful as well.

This volume should continue to find a broad readership outside of Scandinavia. The information included applies to these ferns wherever they grow, and the format and detail of the treatments is uncommon in field guides and coffee table books on plants. It successfully combines the best characteristics of the field guide genre with a rigorous, technical summary of a pteridoflora, and does so beautifully.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

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American Fern Journal 87(2):33–40 (1997)

photo: K. Rasbach

Tadeus Reichstein (1897–1996)

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Professor Tadeus Reichstein was born on July 20, 1897, in Wloclawek (Leslau), at that time in Russian Poland, as the oldest of five boys. His father, Israel, was one of the few Jews that were allowed to study at the technical high school in Leningrad. He worked as a very successful engineer in Kiev. The family of his mother, Gustava Brokman, possessed a flourishing sawmill in Wloclawek. Because of the cruel pogroms in 1905, Israel Reichstein decided to bring his family out of the country and moved to Switzerland, where they found a home in Zurich. From 1907–1914, Tadeus Reichstein was educated (among his brothers and other children from the families of friends) by private teachers

and by his father. Because of the First World War, his family lost all their property in Poland.

In 1916, Tadeus Reichstein passed his final examination in school and was able, in spite of the financially severe conditions, to study at the Swiss Federal Institute of Technology in Zurich. In 1920, he became a chemical engineer and two years later, he was promoted to work with Hermann Staudinger (Nobel prize laureate, 1953). From 1922–1931, Tadeus Reichstein worked in a private laboratory mainly on the volatile compounds of roasted coffee. He developed, together with his old friend Josef von Euw, very sophisticated methods allowing him to make crystalline derivatives. This success was primarily due to his unusual ability in experimenting, a quality that was characteristic for his whole career.

In 1927, Reichstein married. His wife, Henriette Louise née Quarles van Ufford, was fond of music and the arts. She brought another element (devotion to the Muses) into the life of Reichstein, who pursued cultural events with interest during his whole life. They were together for a very long time, and she died in 1993, only three years before her husband.

While working on his thesis at the Institute, Reichstein had the opportunity to meet Leopold Ruzicka (Nobel prize laureate, 1939), and to learn from him modern and efficient methods of analyzing natural products. In 1929, Ruzicka persuaded him to became his assistant at the organic chemical institute of the Swiss Federal Institute of Technology in Zurich. Reichstein also started teaching organic chemistry at about this time. During the years 1931–1938, he and his coworkers found a method to synthesize vitamin C. It was the first successful total synthesis of a vitamin.

After this very successful time in Zurich, he became professor of pharmaceutical chemistry at the university of Basel in 1938. He was director of the institute of organic chemistry from 1946–1960. Owing to his wise leadership, his knowledge, and activities, the institute soon gained a very high international reputation. Here he mainly continued to work on the elucidation and synthesis of corticosteroids, a topic he had started to investigate while still in Zurich. For his findings, he was honored in 1950 by the Nobel Prize.

Tadeus Reichstein had a lifelong fondness for plants and enjoyed greatly cultivating flowers and ferns in his garden. In the late 1950s and mainly after he resigned as the director of the institute of organic chemistry, he became particularly interested in ferns. After his retirement in 1967, he concentrated most of his scientific activities on pteridophytes.

Ferns are the reason why I had the lucky opportunity to know Tadeus Reichstein personally. I remember the many meetings we attended, the excursions he invited me to participate in, the talks we had about ferns in general or about cytotaxonomy and speciation in particular. Although he was more than 70 years old when I first met him, he was mentally and physically in an enviable condition, and great effort was required to keep up with him when he climbed up a steep slope to find some interesting *Asplenium* species. I was later told that he had been a very successful, passionate and well known al-

pinist when he was younger, who climbed many of the high mountains in Switzerland and other European countries.

I remember also with great satisfaction, Tadeus Reichstein as a modest, charming, helpful, kind, and cooperative man. When necessary, he was always ready to help colleagues and scientists and to finance their projects. He supported many indigent persons that came into difficulties during the Second World War and the hard times in the first years after.

His very open, cooperative mind can be imagined easily when looking at the list of shared publications on ferns. Two main lines of research on ferns he followed. One was connected with his "first" profession and consisted of the chemistry of secondary compounds (phloroglucinols) of the fern genus *Dryopteris*. The second concentrated on cytotaxonomy primarily of the genus *Asplenium*, but also on other fern genera.

It was Irene Manton and her students, John D. Lovis and Anne Sleep, who stimulated him greatly in this second focus. He became very conscious of the fact that natural and artificial hybrids are important keystones that allow researchers to learn more about speciation processes. With his extraordinary talent in cultivating ferns (in his private greenhouses in Basel), he carried out large numbers of breeding experiments, which essentially helped to elucidate natural relationships and microevolutionary processes of European, some American, and particularly also Asiatic groups of spleenworts.

The results of the many projects are shown in his long list of publications. Whenever I visited him, he showed me with much enthusiasm the ferns in his garden and the hundreds of pots in the greenhouses with the experimental plants (parent species and hybrids). Tadeus Reichstein was scientifically active nearly until the end of his very long and impressive life (he passed away on August 1, 1996, at the age of 99). In his final years, he was mainly occupied with writing down the many important results of his later experiments, mostly for "Flora Iranica". In 1996, I met him for the last time. Although his physical condition was not good any more, he was mentally very present, and sitting together in his home with tea and cake we had a stimulating talk about ferns and fern research. I will always remember him as a fatherly and generous friend, from whom I learned a lot about ferns and a lot about humanity.

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SEM Studies on Vessels in Ferns. 4. *Astrolepis*

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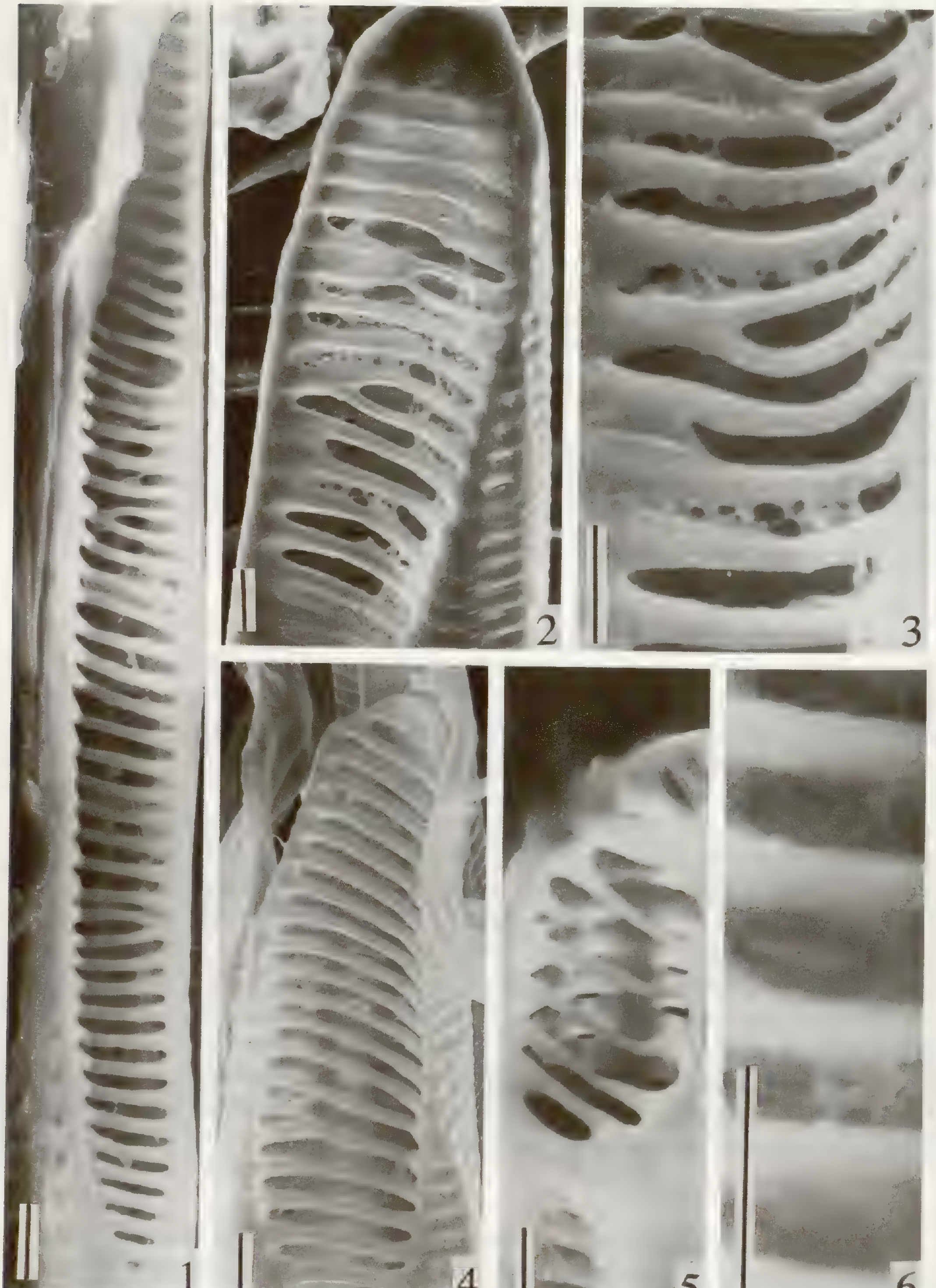
ABSTRACT.—By means of scanning electron microscopy, the nature of vessels is described for roots and rhizomes of *Astrolepis sinuata* (Lag. ex Swartz) D.M. Benham & Windham [= *Notholaena sinuata* (Lag. ex Swartz) Kaulf.], commonly placed in Pteridaceae. In both roots and rhizomes, end walls are variously distinct from lateral walls; a few perforation plates are quite elongate with numerous bars, but most are of medium or short length with fewer bars. Pit membrane remnants are generally sparse in the perforation plates. Lateral wall perforation plates are present; some may represent a multiplicity of facets at the tip of a tracheary element. Lateral walls may have intact pit membranes or perforations. No tracheids were identified; apparently all tracheary elements are vessel elements. The abundance of relatively short end walls with few bars on perforation plates may be correlated with rapid rates of water conduction during the short growing season of this fern, which occupies outcrops in arid parts of the southwestern United States and Mexico.

Scanning electron microscopy (SEM) offers a highly effective method of demonstrating the presence of vessels, because with this method pores large enough to constitute perforations can be seen. We have been attempting to describe the nature and extent of vessels in ferns by means of SEM (Carlquist et al., 1997; Carlquist and Schneider, in press; Schneider and Carlquist, 1997. Our studies have taken as a point of departure the studies of White (1962, 1963), in which end walls appreciably different from lateral walls were recorded in tracheary elements of particular ferns. White (1962) suspected that some of these tracheary elements might be vessels. *Astrolepis sinuata* was a species for which White (1962) figured tracheary elements that have "end plates" different in their patterns from lateral wall pitting. Light microscopy cannot reveal presence of pores in pit membranes of ferns, and thus White's conclusions on vessel presence on the basis of light microscopy were speculative except where clear presence of vessels is concerned. White (1961, 1962) accepted the presence of vessels in *Pteridium* and *Marsilea*. Because of the inability of light microscopy to resolve perforations, earlier literature has reports that vessels are present in some ferns other than *Pteridium* (Gwynne-Vaughan, 1908); these reports were contradicted by Bancroft (1911) and Duerden (1940).

In extending White's observations, we can confirm that there are clear instances of vessels in which scalariform perforation plates lacking any vestiges of pit membranes are present. Some of the vessels of *Pteridium* conform to this pattern (Carlquist & Schneider, in press), although some vessels in that species retain pit membranes that are porose; these porosities qualify such tracheary elements as vessels. In some ferns, only porose pit membranes occur on end walls; this is true of *Woodsia obtusa* (Sprengel) Torrey (Carlquist et al., 1997). Perforations of this sort on lateral walls of vessels were reported in



FIGS.1–6. SEM photographs of tracheary elements from macerations of roots of *Astrolepis sinuata*, showing perforation plates or portions of perforation plates; concave conformations in some of these plates are artifacts. 1) Long perforation plate with few pit membrane remnants between bars. 2) End wall of medium length, with some intact pit membranes and with perforations that have variously porose pit membranes; imperforate lateral wall pitting, lower right. 3) Portion of a perforation plate, showing some pit membrane remnants in which small pores are evident. 4) End

Pteridium (Carlquist and Schneider, in press) and in *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997). In the latter two genera, perforations free from pit membranes as well as perforations that retain pit membranes were observed (Schneider and Carlquist, 1997). Thus, a range of expressions in vessel presence is now known to occur in ferns.

The diverse patterns of vessel presence and morphology discovered thus far by means of SEM suggest that vessels are much more widespread in ferns than hitherto suspected. In turn, phylogenetic and physiological implications are raised. Are vessels polyphyletic in ferns, and if so, have they originated many or few times? Do the presence of vessels in particular ferns and the morphology of those vessels relate to the ecological regimes to which those species are adapted, respectively? What is the physiological significance of porose pit membranes, e.g., can air embolisms traverse such pores? Are porose membranes better than imperforate membranes at conduction of water in xylem? Why do some ferns have porosities in pit membranes of lateral walls, and what adaptive significance do lateral-wall perforation plates have? Although we cannot answer all of these questions on the basis of our SEM studies, we believe we can provide a data base that can contribute to an improved understanding of phylesis and physiology of fern xylem. *Astrolepis sinuata* is a fern of particular significance with regard to ecological significance of vessels, because it occurs in dry areas (usually in rock outcrops) from western Texas to southeastern California and to South America (Lellinger, 1985).

Materials and Methods

Our material of *Astrolepis sinuata* was cultivated in the Botanic Garden of the University of California at Berkeley. This plant was derived from a plant cultivated at the New York Botanic Garden, which was grown from a plant collected in the wild by J. Beitel and J. Mickel (s.n.), 23 Jan 1989, on a hill called Las Cruces at Tlacolulu, at the intersection of Route 190 with Route to Macuilxochitl, east of Oaxaca; on steep rocky hill, 1600 m, det. by A. Smith. Living material was sent to us; we preserved portions in 50% aqueous ethanol. Macerations of roots and stems were prepared with Jeffrey's Fluid, stored in 50% aqueous ethanol, spread over the surface of aluminum stubs, sputter-coated, and examined with a Bausch and Lomb Nanolab SEM. Paraffin sections were prepared according to the usual techniques, mounted on glass slides, and stained with Northen's modification of Foster's tannic acid-ferric chloride method (Johansen, 1940). These sections were useful in seeing the vascular structure of the root and stem with light microscopy and thereby offering data that could be compared to findings made with SEM. Our earlier work with *Pteridium* (Carlquist and Schneider, in press), in which we examined both

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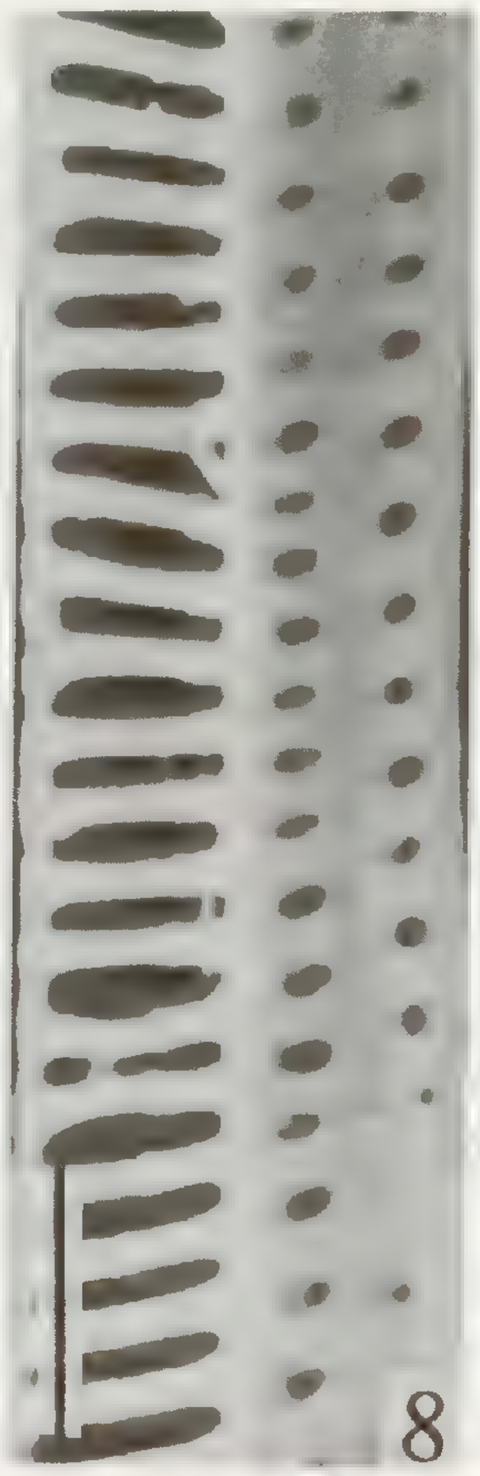
wall of medium length, in which very few pit membrane remnants are present; imperforate lateral wall pitting at right. 5) Short end wall with few bars on perforation plate and no pit membrane remnants. 6) Weblike pit membranes in perforations of an end wall. Scale bars = 10 μm.



FIGS.7–11. End walls (Figs. 7–9) and lateral walls (Figs. 10–11) from tracheary elements of rhizome of *Astrolepis sinuata*. 7) Portion of perforation plate with pit membrane remnants in most of the perforations. 8) End wall perforation plate with few remnants in perforations, left, and two narrower perforation plates, right. 9) Short end wall in which the perforation plate has few, wide bars. 10) Lateral walls of two adjacent tracheary elements; most of the lateral wall faces of the element at left lack pores, but the lateral wall of the element at right is highly porose. 11) Two

sectioned and macerated xylem with SEM, convinced us that the data obtained with the two methods are identical. Because fern tracheary elements have hard secondary walls and break excessively when sectioned longitudinally, we have elected to use macerations as the source of observations in the present paper.

We judge all of the tracheary elements figured to be quite mature, because stem portions selected for maceration were well distal to the apical meristem, and root portions selected for maceration were adjacent to the rhizome. Thus, we believe that tracheary elements with porose pit membrane remnants in perforations are comparable in degree of maturity to tracheary elements with perforations free from pit membrane remnants.

Results

Tracheary elements of roots (Figs. 1–6) are likely all vessels, because we could not find any tracheary elements clearly lacking porosities in end walls. The perforation plates of vessels, however, range widely in morphology. Some plates are very long with numerous bars and only a few remnants of primary wall material (Fig. 1). Some end walls are shorter (Fig. 2), and feature pits without pores (Fig. 2, top and bottom) as well as perforations in which lysis has produced small to large pores (Fig. 2, center). Perforations often feature series of circular to oval pores, with various degrees of coalescence to form large holes (Fig. 3). One can find all sizes of pores, ranging from some nearly as large as perforations to some that barely be resolved with our equipment (Fig. 3). In some perforation plates of medium length, remnants of pit membranes are entirely absent (Fig. 4). Some perforation plates have very few and narrow bars (Fig. 5). All of the perforation plates in Figures 1–5 are markedly wider than the relatively narrow cell faces presented by lateral wall pitting (Fig. 2, right; Fig. 4, right). In some end walls, however, delicate weblike pit membrane remnants were noted (Fig. 6). Although pores in lateral wall pits of tracheary elements of roots are not figured, we noted a few instances of this phenomenon.

In tracheary elements of rhizomes (Figs. 7–11), there is a range in perforation plate morphology similar to that seen in roots. The end wall shown in Fig. 7 shows a perforation plate similar to lateral wall pitting: the bars between the perforations are wide, but most of the perforations are relatively free from pit membrane remnants. At right in Fig. 7 is a smooth, unpitted portion of the vessel wall. Although macerations are not suitable for determination of contacts between particular cell types, we believe that this smooth wall represents a vessel-parenchyma interface because parenchyma has inconspicuous primary pit fields that do not have a scalariform disposition. Scalariform lateral wall pitting is therefore a reliable indicator that a tracheary element cell face was adjacent to a similar face of another tracheary element. Parenchyma cells

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lateral walls of a tracheary element, showing prominent perforations, relatively few pit membrane remnants. Scale bars=10 μm.

were observed in our macerations, but have not been figured here. The vessel element in Fig. 8 shows similarity between an end wall with a wider perforation plate at left and two narrower perforation plates at right. One could say that in this vessel element, there are several perforation plates that contact perforation plates of other vessel elements superposed or even lateral to the one shown. The distinction between a perforation plate and lateral wall pitting disappears in such a vessel element. Also present in the rhizome are some vessel elements with clear differentiation between end walls and lateral walls, as in the vessel element shown in Fig. 9 (lateral walls not shown in this figure, but similar to lateral walls of Fig. 10). The perforation plate consists of few and wide bars.

Lateral walls of rhizome tracheary elements are shown in Figs. 10, 11. In Fig. 10, there are two tracheary elements. In the one at left, relatively few pores penetrate the pit membranes. More numerous and wider openings in the pit membranes occur in the lateral wall shown for the tracheary element at right. In Fig. 11, absence of pit membrane remnants is notable; one must call these surfaces perforation plates on this account. Thus, in *Astrolepis sinuata* there are all degrees of cell wall openings from imperforate lateral wall pits to perforation plates.

Discussion and Conclusions

In both roots and rhizomes, perforation plates in *Astrolepis sinuata* range from little differentiated, with abundance of porose pit membrane remnants, to scalariform perforation plates with few bars and no pit membrane remnants. There are also lateral perforation plates, although not all lateral walls facing other tracheary elements have porose pit membranes: some of these lateral walls retain intact pit membranes. All tracheary elements appear to have perforation plates, and no tracheids were identified with certainty. All of these features can be found in *Pteridium* (Carlquist and Schneider, in press) as well as *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997). Tracheary elements of *Woodsia obtusa* (Carlquist et al., 1997) have no lateral perforation plates, and the end walls are relatively little differentiated as perforation plates; pit membrane remnants are present, and are moderately porose. However, these features should not be considered as differences between the genus *Woodsia* and the other genera. Other species of *Woodsia,* such as *W. ilvensis* (L.) R. Brown, very likely when studied with SEM will prove to have perforation plates as specialized as those of *Pteridium,* if not more so, judging from the light microscope studies of White (1962). Parenchyma-vessel interfaces of *Astrolepis sinuata* are notable for absence of pitting; examination of transections of the meristeles shows that parenchyma is relatively abundant within the xylem of this species.

The degrees of specialization of perforation plates in the ferns we have studied to date are not related to taxonomic position. The development of vessels in ferns, like that in monocotyledons, seems likely to relate to ecology. As yet, the number of ferns we have studied is insufficient to develop an outline of

the relationship between types of tracheary elements and ecology. We believe that such a relationship, as with monocotyledons (Carlquist, 1975) is likely. As patterns of relationship between xylem structure and ecology become clearer, implications for physiology of fern xylem will emerge. Thus, our survey of fern xylem can offer the basis for valuable advances in understanding of fern physiology.

We have begun this series of papers with studies of ferns in which vessel presence could likely be demonstrated when studied with SEM, rather than ferns in which xylem might prove to consist of tracheids only. Certainly *A. sinuata* qualifies as a xeric fern. Therefore, vessels with simplified (fewer bars, absence of pit membranes), as found in this species, seem to correlate with conduction of larger water volumes during the short season during which soil moisture is abundant.

Tracheary elements with a greater degree of differentiation of end walls from lateral walls might be expected in roots, rather than rhizomes of ferns, judging from the data of White (1962; see also Carlquist et al., 1997). Although observation of SEM preparations does not permit development of quantitative data on this point, we note that in all of the ferns we have studied to date, vessel elements in both roots and rhizomes have a range of morphology from tracheid-like to moderately specialized. Future studies are needed to establish whether the degrees of specialization of perforation plates in roots as compared to rhizomes are about the same within a given fern species, or whether, as in Cheadle's (1942) study of monocotyledons, there is greater specialization in perforation plates of roots as compared to those of stems.

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Palynological Study of *Cheilanthes* and *Astrolepis* (Pteridaceae) Species from Northwestern Argentina

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ABSTRACT.—The spore morphology of *Cheilanthes* and *Astrolepis* species from northwestern Argentina was analyzed using light microscopy and scanning electron microscopy. Three patterns of sporoderm structure were found: two exclusive and different structures in *A. sinuata* and the *C. squamosa* group, and a typically cheilanthoid structure with diverse sculptures in the rest of the species of *Cheilanthes*, i.e., *C. bonariensis, C. buchtienii, C.* cf. *cucullans, C. obducta,* and *C. volcanensis.* Sporophytic features such as hairs with "tongue and groove" wall cells and dixylic vascular strands are also mentioned in *C. obducta.* Similar spore characteristics are correlated with sporophyte morphology in some of the recognized groups. Spore-size differences, abnormalities in spore type, abortion and number of spores per sporangium (16, 32) suggest polyploidy and/or apogamy in some specimens of *C. buchtienii, C. obducta,* and *C. squamosa,* and in all samples of *A. sinuata* and *C. bonariensis.*

The present paper is the second in a series of studies on cheilanthoid ferns preliminary to an ongoing project, "Atlas of Spores of Pteridophyta of Northwestern Argentina" (Morbelli, in prep.). Perispore structure and stratification offer some interesting substructures and variations. Previous papers (Ranker, 1989; Tryon and Lugardon, 1991) have shown that detailed analysis of the exospore and perispore can provide valuable information in cheilanthoid ferns. The aim of these papers is to characterize each species of the region from the palynological point of view and to assess whether spore morphology is useful in the systematics of the group. When necessary, sporophytic features are included for comparative analysis of species groups.

METHODS

In the first part of this study (Morbelli and Michelena, 1989), the following species of *Cheilanthes* were treated: *C. marginata* Kunth, *C. micropteris* Sw., *C. myriophylla* Desv., *C. notholaenoides* (Desv.) Weath., *C. pilosa* Goldm., *C. poeppigiana* Mett. ex Kuhn, and *C. pruinata* Kaulf. The present paper includes the species previously recognized as part of *Notholaena* (Tryon, 1956; de la Sota, 1977) and later placed into *Cheilanthes* (Tryon and Tryon, 1982) and *Astrolepis* (Benham and Windham, 1992). The following taxa were analyzed: *A. sinuata* (Lag. ex Sw.) D.M. Benham & Windham, *C. arequipensis* (Maxon) R.M. Tryon & A.F. Tryon, *C. bonariensis* (Willd.) Proctor, *C. buchtienii* (Ro-

senst.) R.M. Tryon, *C.* cf. *cucullans* Fée (de la Sota and Ponce, 1992), *C. obducta* Mett. ex Kuhn, *C. squamosa* Hook. & Grev. and *C. volcanensis* de la Sota.

Samples of spores and observations of sporophytes were taken from herbarium specimens (LIL,LP,SI). Spores were treated with 3% hot sodium carbonate for 2 min in order to preserve the perispore before acetolysis (Erdtman 1960). Spores were mounted in glycerine-jelly on glass slides; cover slips were sealed with parafin. Slides are accessioned in the Palynology Area, Botany Department of the Facultad de Ciencias Naturales y Museo de La Plata. Olympus BH2 and BHB microscopes were used for the light microscopy (LM) analysis.

For scanning electron microscopy (SEM), the material was treated with hot sodium carbonate (3%) for 2 min, washed in distilled water, and suspended in ethanol (96%), prior to mounting on stubs and examination using a JEOL JSM-35 CF instrument. Wall fractures for studying ultrastructure with SEM were made with ultrasonic equipment. Then the material was transferred through capillary tubes to acetate plates, to which spores adhered. Later the material was sputter-coated with gold.

Measures of the number of spores per sporangium were obtained by placing a single, mature sporangium in a drop of water-glycerine (50%) medium, and heating gently to crush the sporangium. The large-sized spores were measured and cited separately.

For descriptions of spore morphology, the terms proposed by Tryon and Tryon (1973) and Morbelli and Michelena (1989) were used. The classification of Tryon and Tryon (1982) was adopted for recognition of species groups.

Results

In the following descriptions, spore dimensions and sizes of structures are given as the range followed by the mean in parentheses, except where variation was negligible. The abbreviations De and Dp refer to the spore diameters in equatorial and polar views, respectively.

1. **Astrolepis sinuata** (Lag. ex Sw.) D.M. Benham & Windham.—*Cheilanthes sinuata* (Lag. ex Sw.) Domin.—Fig. 4E–H.

Description.—Spores: trilete, with triangular outline in polar view, straight sides and rounded corners; De 53.0–98.0 (79.0) μm, Dp 78.3–85.5 (82.8) μm. Laesura arms: 24.3–38.7 (32.7) μm long and 2.7 μm high, straight, tenuimarginate. Exospore: yellow, 1.1–4.6 (2.5) μm thick, rugulate, 2-layered. Perispore: pale brown to yellow, 0.7–1.3 (0.8) μm thick, 1-layered; folded. Folds short, 0.9–4 (2.7) μm high; conic with rounded apex in section; densely distributed, resulting in a rugulate surface. Folds sometimes radially oriented at the proximal pole and concentrically distributed at the distal one. Background sculpture: mixed (granules, baculae, gemmae, and echinae) sparsely and randomly distributed, 1.3-1.6 (1.4) μm high.

Comments.—In all of the material studied, ripened spores were found, but

TABLE 1. Spore numbers per sporangium and spores sizes for various spore types in the specimens of *Astrolepis sinuata* examined in the present study. Spore sizes are listed as ranges, followed by means in parentheses.

Specimen number	Types of spores and sizes (μm)				Number of spores per sporangium
	Trilete	Monolet	Inter-mediate	Alete	
Castillón s.n. (LIL)	53–96 (71)	74.4–93.6 (84)	76.8	—	32
Budin 11818 (LIL)	62–96 (82)	—	—	125–127 (126)	32
Lillo 3860bis (LIL)	65–98 (84)	84	106	110.4	32; 16

abnormalities, such as double, monolete, intermediate, and alete spores also were found within the same samples. The spores per sporangium were 32 and in 1 specimen were 32 and 16 (see Table 1). Larger sizes in spore diameters than those given by Benham (1995: 120) were estimated in all the specimens of the study area.

SPECIMENS EXAMINED.—Jujuy, Maimará, Hualchin, *Budin 11828* (LIL); Tucumán, Rosario de la Frontera, *Lillo 3860bis* (LIL); Trancas, Las Higueritas, *Legname & Cuezzo 4632c* (LIL); Catamarca, Ambato, El Potrero, Quebrada de Los Nacimientos, *Castillón* s.n. (LIL 16998).

2. **Cheilanthes arequipensis** (Maxon) R.M. Tryon & A.F. Tryon.—Fig. 1A–C.

DESCRIPTION.—Spores: trilete, with triangular outline straight sides and round corners in polar view. De 48.6–56.7 (51.7) μm; Dp 45.0–51.3 (47.9) μm. Laesura arms: straight, 20.7–36.0 (27.7) μm long, crassimarginate with ends frequently bifurcate. Exospore: yellowish to brown, 2.2–2.7 (2.5) μm thick, 2-layered, verrucate. Verrucae 1.3–1.8 (1.6) μm high, 3.6–4.9 (4.4) μm in diam.; smaller at the proximal pole (2.8–4.3 (3.6) μm in diam). Some verrucae fused at the distal pole. Perispore: hyaline, 0.4–0.8 (0.6) μm thick, strongly attached to the exospore, 1-layered, granular.

COMMENTS.—Monolete spores were found in *Cristóbal & Türpe 13* (LIL) with two kinds of exospore sculpture: smooth and verrucate. The verrucae are small.

SPECIMENS EXAMINED.—Jujuy, Tilcara, Cerro Negro, *Cristóbal & Türpe 13* (LIL); Mina Aguilar, *Broucher* s.n. (LIL 333888).

3. **Cheilanthes bonariensis** (Willd.) Proctor.—Fig. 2A–C.

DESCRIPTION.—Spores: trilete, with circular to subcircular outline in polar view. De (a) 63.9–80.1 (70.4) μm; De (b) 69.7–80.1 (72.7) μm; Dp 67.5 μm. Laesura arms: straight, 30.6–39.6 (33) μm long, 3.6 μm high, 1.7 μm thick, reaching the equator, crassimarginate. Exospore: brown, 1.5–3.1 (2.2) μm thick, 2-layered, smooth. Perispore: pale yellow to yellow, 1.8–4.5 (2.8) μm thick, strongly attached to exospore, 3-layered; inner layer thin; middle layer well developed, thick, composed of threads fused in several levels; outer layer thick, with undulate margin; closed structure (Morbelli and Michelena, 1989),

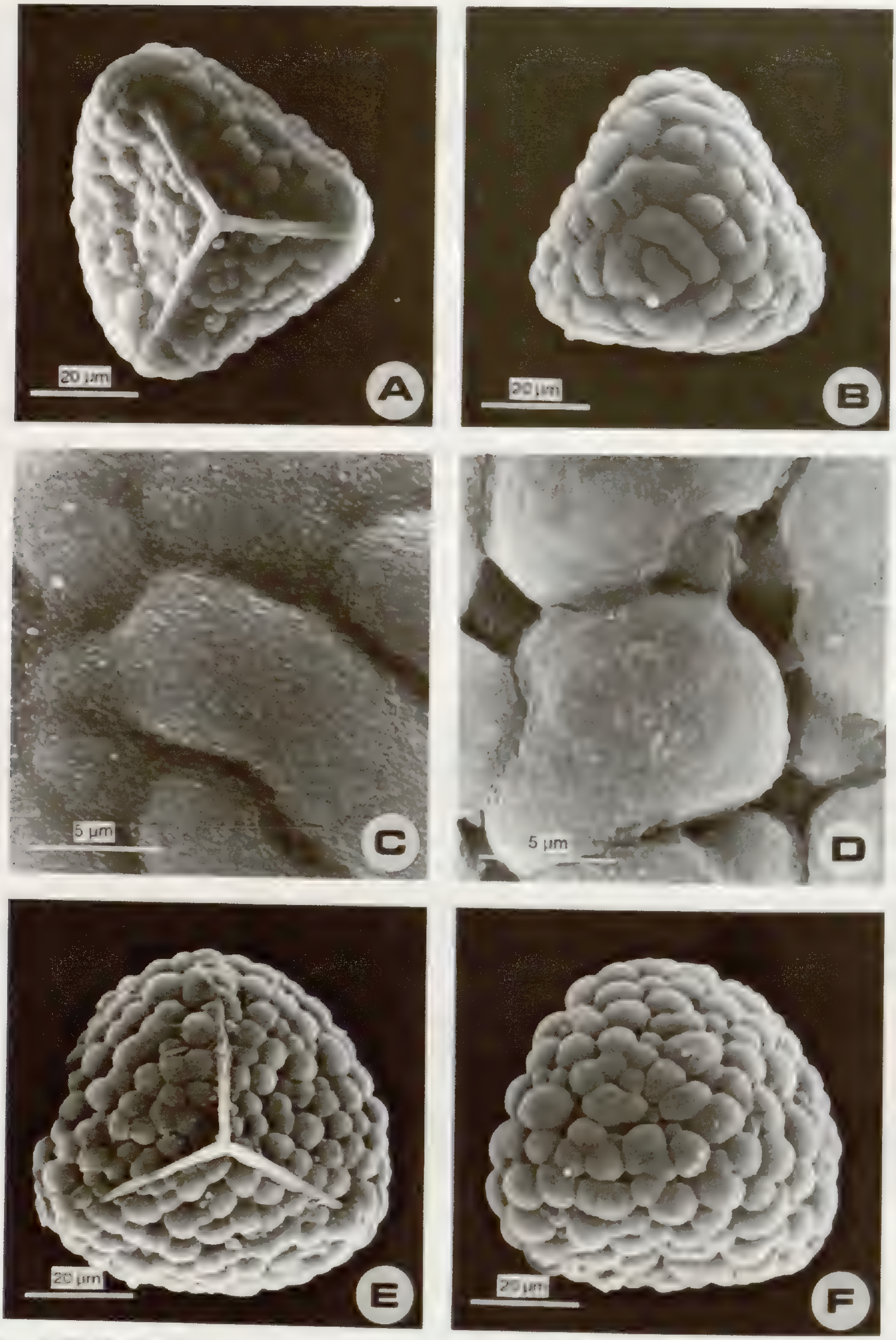

FIG. 1. A–C) *Cheilanthes arequipensis*. A) Proximal view. B) Distal view. C) Surface detail, showing the verrucate exospore and the granulate perispore on it (*Cristóbal & Türpe 13*, LIL). D–F) *Cheilanthes squamosa*. D) Surface detail, showing round verrucae of the exospore and the scabrate perispore on it. E) Proximal view. F) Distal view (*Sleumer & Vervoorst 2526*, LIL).

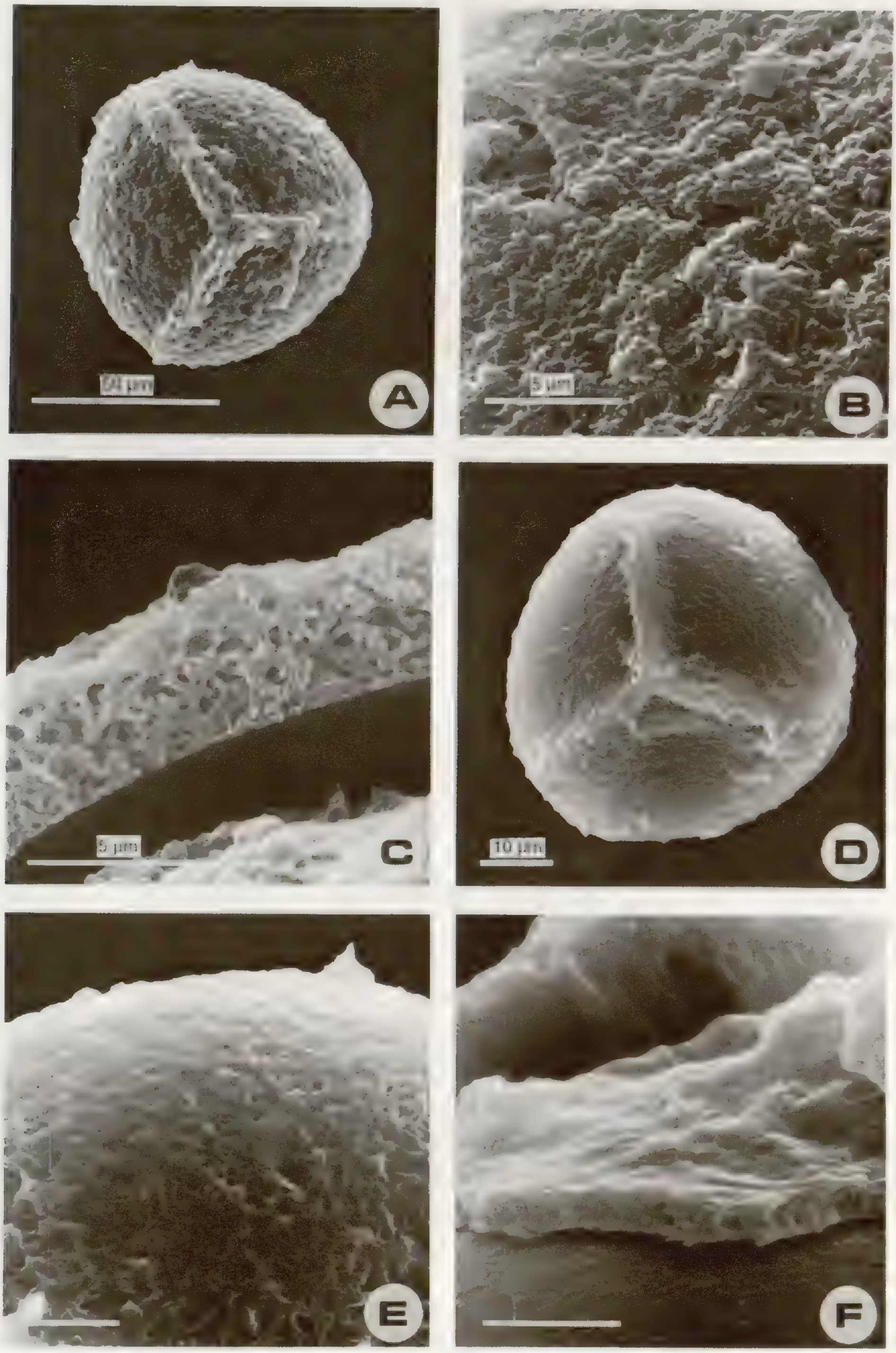

FIG. 2. A–C) *Cheilanthes bonariensis*. A) Proximal view. B) Surface detail, showing the rugulate perispore. C) Fractured perispore section, showing the thick middle complex layer with several levels of fused threads (*Sleumer & Vervoorst 2821*, LIL). D, E, F) *Cheilanthes* cf. *cucullans*. D) Proximal view. E) Surface detail, showing the rugulate perispore. F) Fractured perispore section, showing the thin perispore with a narrow middle layer (*Palací 34*, LP).

rugulate. Rugae low 0.25–0.5 (0.4) μm high, densely packed, placed in a concentric pattern. Background sculpture: granulate.

COMMENTS.—The number of spores per sporangium was both 32 and 16 in all the specimens studied. These data support earlier observations that *C. bonariensis* is a triploid, apomictic species (Knobloch et al., 1973). Well-developed trilete spores (70–80 μm De), were found in *Sleumer and Vervoorst 2821* (LIL), but few monolete spores (115.2 μm DE), were noted as well. In *C. bonariensis*, as in other low-ornamented spores, the perispore covers and modifies the laesura, making it more evident and thick when seen in surface view. Some specimens showed the perispore cracked after acetolysis. There is no agreement between the features of the spores in our samples and the ones studied by Naab (1987). In our study, the perispore was strongly attached to the exospore. However our samples show strong similarities to materials from Bolivia studied by Tryon and Lugardon (1991).

SPECIMENS STUDIED.—Jujuy, Humahuaca, *West 6309* (LIL); Salta, Caldera, Potrero del Castillo, *Sleumer & Vervoorst 2821* (LIL 395520); Catamarca, Andalgalá, *Sleumer 1846* (LIL); Tucumán, Tafí, Siambón, *Lillo 1040* (LIL); Chicligasta, de El Bolsón a la Cascada, *Meyer 18188* (LIL); Amaicha, El Molle, unknown coll. s.n. (LIL 16924).

4. **Cheilanthes buchtienii** (Rosenst.) R. M. Tryon.—Fig. 3A–D.

DESCRIPTION.—Spores: trilete, with triangular outline in polar view. De (a) 56.7–61.2 (58) μm, De (b) 60.3–68.4 (63) μm, Dp 47.7–52.2 (49.9) μm. Laesura arms: 22.5–27.0 (24.7) μm long, 3.6 μm high, reaching the equator, tenuimarginate or indistinctly marginate. Exospore: yellow, 1.3–2.0 (1.6) μm thick, 2-layered, smooth. Perispore: brown, 1.8–3.6 (2.5) μm thick, closely attached to the exospore, 3-layered; inner layer thin; middle layer well developed, composed of fused threads in several levels; outer layer thin; closed structure (Morbelli and Michelena, 1989); cristate to cristate-reticulate. Cristae 1.7–2.7 (1.9) μm high. Background sculpture: perforate.

COMMENTS.—The specimen *Barkley 19AR 649* (LIL) contained 64-, 32- and 16-spored sporangia, with evident differences in spore-sizes. However, there was no significative variation in spore-sizes in *Cuezzo and Legname 2257* (LIL), which nevertheless has 64- and 32-spored sporangium. Different specimens show cristate patterns of varying height. In this case, as in cristate-reticulate spores, the perispore covers and modifies the laesura, making it unclear or indistinguishable when seen in surface. Sometimes the perispore is cracked and detached after acetolysis. There are spores with different wall color, perispore thickness, and complexity, which might reflect differences in degree of maturation. Contrary to Michelena (1989), big differences were not found between the spores of *C. buchtienii* and its variety *ventanensis* (Weath.) Capurro. Contrary to Naab (1987), we found that the perispore was strongly attached to the exospore.

SPECIMENS EXAMINED.—Salta, Santa Victoria, Santa Victoria, *Meyer 4830* (LIL); Tucumán, Trancas, Las Burras, Río Las Burras, *Cuezzo & Legname 2257* (LIL); Catamarca, Ambato, El Rodeo, *Barkley*

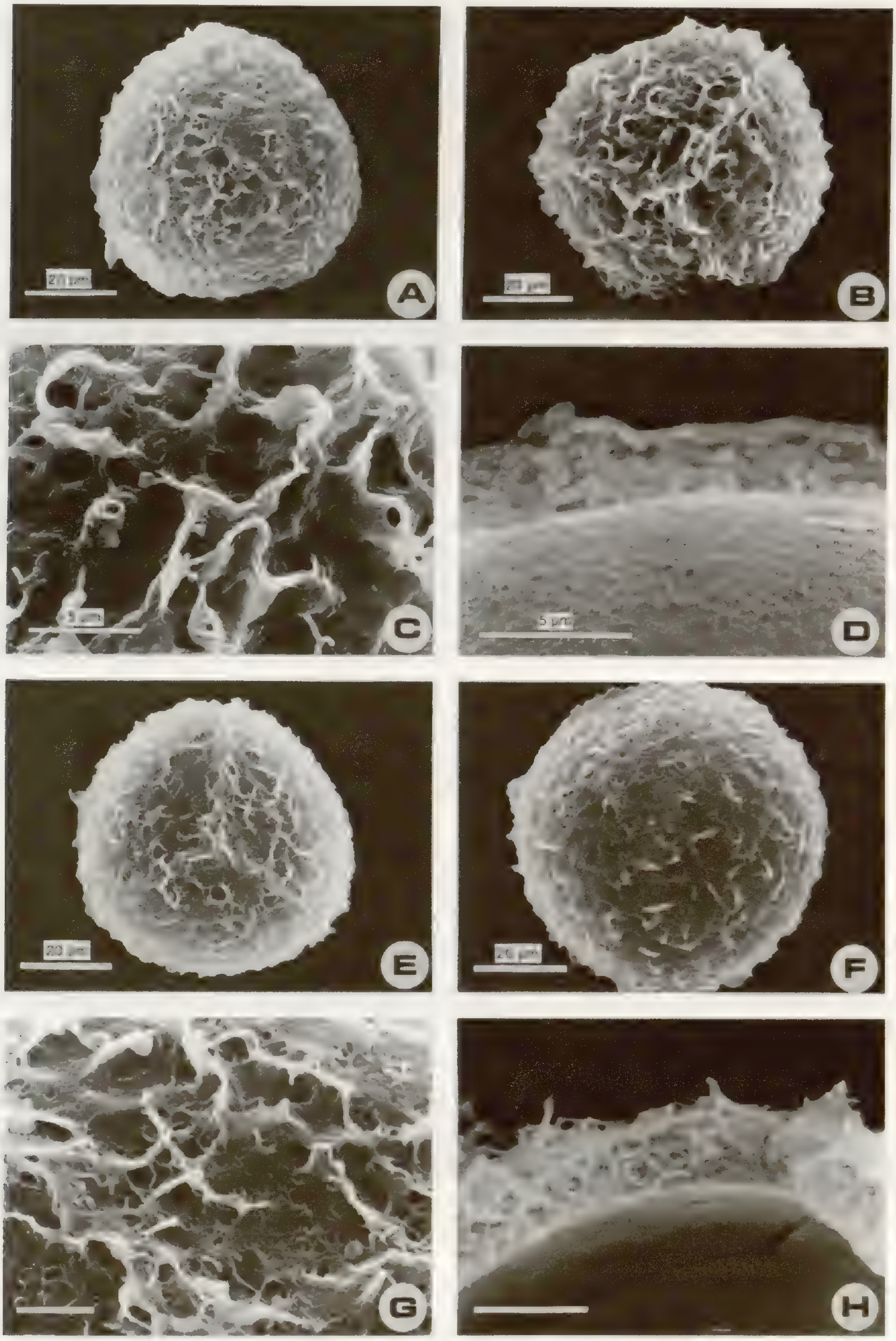

FIG. 3. A–D) *Cheilanthes buchtienii*. A) Distal view. B) Proximal view. C) Surface detail, showing a cristate to cristate-reticulate perispore. D) Fractured perispore section, showing the thick middle layer with several levels of fused threads (*Meyer 4830*, LIL). E-H) *Cheilanthes volcanensis*. E) Proximal view. F) Distal view. G) Surface detail, showing the cristate sculpture of the perispore. H) Fractured perispore section, showing the complex middle layer with several planes of thread junctions (*Lillo 3460*, LIL).

19AR 649 (LIL); Santiago del Estero, Guasayán, Tazana, *Pierotti* s.n. (LIL 109887); La Rioja, Quebrada de Los Noques, Malanzán, *Vera de Gandio* s.n. (LIL); San Luis, Junín, Piedra Blanca, *Digilio & Grassi 2178* (LIL).

5. **Cheilanthes cf. cucullans** Fée.—Fig. 2D–F.

DESCRIPTION.—Spores: trilete with triangular outline in polar view. De 46.2–52.5 (50.4) μm. Laesura arms: straigth, 25–26.3 (25.6) μm long, 2.5 μm high, tenuimarginate, reaching the equator. Exospore: not seen with ligth microscope, smooth in SEM view. Perispore: 0.7–1.2 μm thick, strongly attached to the exospore, 3-layered; inner layer thin; middle layer low, composed of threads fused in few levels; outer layer thin; closed structure (Morbelli and Michelena, 1989); rugulate. Rugulae low, 0.2–0.5 μm high. Background sculpture: echinulate-punctate. Some spores with scarcely differentiated threads plus echinula.

COMMENTS.—The presence of *C. cucullans* in Argentina is somewhat unusual because this species is otherwise restricted to Mexico and Guatemala. The specimen studied appears morphologically identical to those of North and Central America. The species was cited as present in South America by de la Sota and Ponce (1992). The outer layer of the perispore is so thin that it scarcely covers the structure below. Spores from the scant material were not examined with LM, thus exospore thickness and exospore/perispore color could not be estimated. It is necessary to point out the similarity between the spores of *C. cf. cucullans* and the closely related *C. notholaenoides*, the latter studied by Morbelli and Michelena (1989).

SPECIMENS EXAMINED.—Salta, La Viña, *Palací 34* (LP).

6. **Cheilanthes obducta** Mett. ex Kuhn.—Fig. 4A–D.

DESCRIPTION.—Spores: trilete, with triangular to triqueter outline in polar view. De: 56.7–61.2 (58.4) μm; Dp: 58.5–66.6 (63.1) μm. Laesura arms: straight, 20.7 36.0 (25.0) μm long, 3.6–6.3 (5.1) μm high, tenuimarginate, reaching the equator. Exospore: yellow to brown, 1.35 μm thick, 2-layered, smooth. Perispore: pale yellow, 0.2–0.6 μm thick, 3-layered; inner layer thin; middle layer low; outer layer thin; closed structure (Morbelli and Michelena, 1989); echinate-baculate. Echina/baculae 0.9–1.8 (1,3) μm high with forked bases. Background sculpture: granular. Granules sometimes grouped.

COMMENTS.—The description was based on *de la Sota 1241*, in which the presence of echinae or baculae on the perispore surface was not a constant feature. Spores in *Türpe et al. 4634* are larger than in the others samples. They are De 59.4–66.7 (63.2) μm; Dp 67.5–82.8 (72.9) μm, and the sporoderm is thicker as well; this may reflect a ploidy difference. The same material has abnormalities, such as double and aborted spores. It also contains 32-spored sporangia, which indicates that is probably apomictic. Three types of perispore sculpture have been recognized in different specimens: granular (*Morello and Cuezzo*

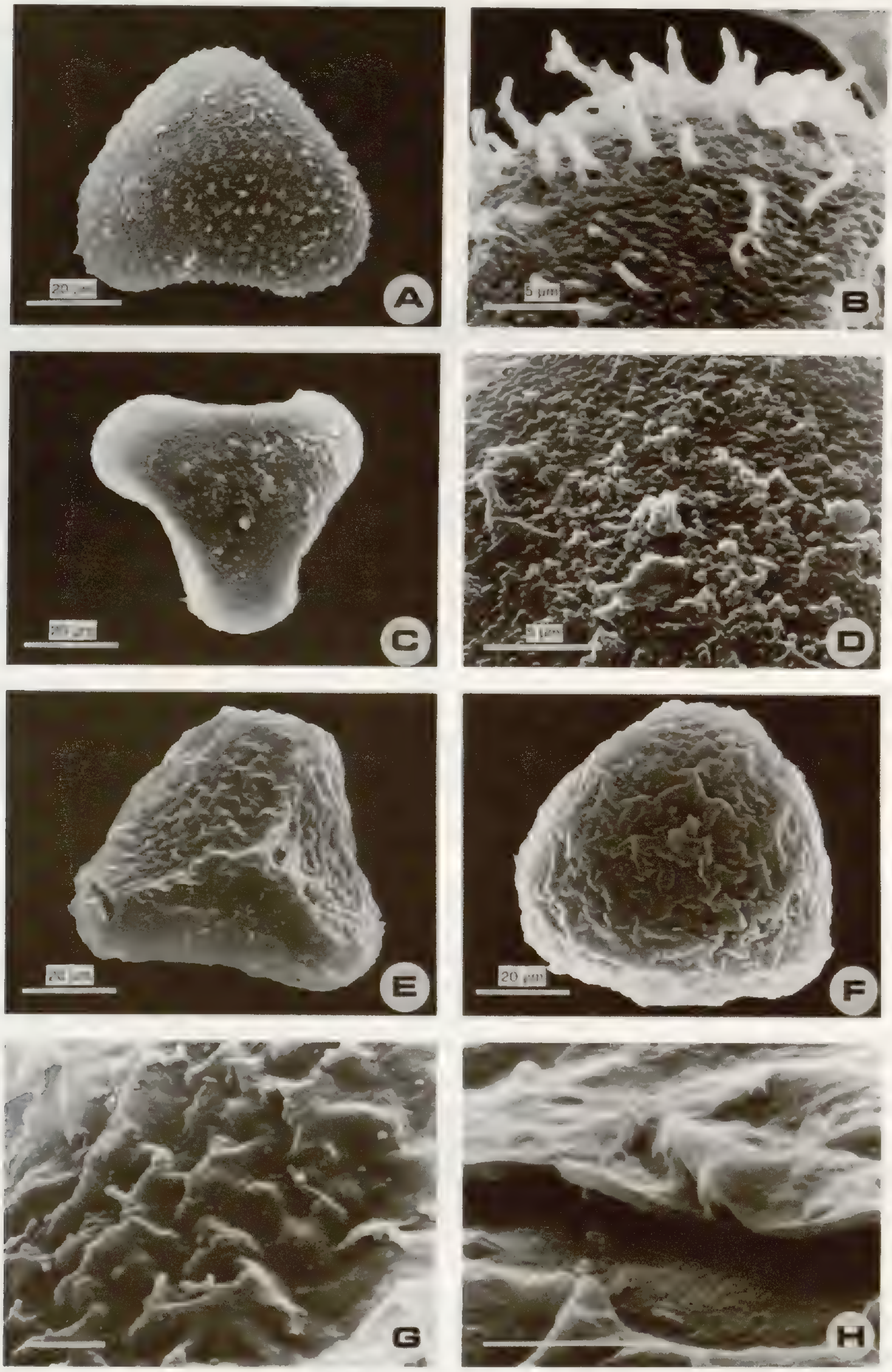

FIG. 4. A–D) *Cheilanthes obducta*. A) Distal view. B) Surface detail, showing the granulate-baculate perispore (*de la Sota 1241*, LIL). C) distal view. D) Surface detail, showing a granulate perispore (*Morello & Cuezzo* s.n., LIL 505245). E–H) *Astrolepis sinuata*. E) Proximal view, showing the folded perispore. F) distal view. G) Surface detail, showing short folds of the perispore. H) Fractured perispore section, showing the thin perispore, and the folds and granulate sculpture of the exospore beneath (*Lillo 3860bis*, LIL).

s.n., LIL 505245), rugulate (*Castellanos* s/n, LIL 325315) and equinate-baculate (*Türpe et al. 4634,* LIL).

SPECIMENS EXAMINED.—Jujuy, Santa Bárbara, Laguna La Brea, *Türpe et al. 4634* (LIL); Salta, San Martín, Aguaray, *de la Sota 1241* (LIL); Formosa, Bermejo, al N de Pozo de Maza, *Castellanos* s.n. (LIL 325315); Tucumán, Capital, Barranca Colorada, *Venturi 846* (LIL); Catamarca, Sierra del Alto, *Morello & Cuezzo* s.n. (LIL 505245); Ambato, Quebrada de Los Nacimientos, *Castillón* s.n. (LIL 17007); *La Rioja,* Sierra Alta, *Castellanos* s.n. (LIL 96052).

7. **Cheilanthes squamosa** Hook. & Grev.—Fig. 1D–F.

DESCRIPTION.—Spores: trilete, with triangular outline in polar view, convex sides and round angules; De 59.4–66.7 (63) μm. Laesura arms: 22.5–28.8 (26.4) μm long, straight, elevated. Exospore: 6.2–7.2 (6.4) μm thick, 2-layered, verrucate. Verrucae 5.5–6.3 (5.7) μm high, 7.2–10.8 (9.2) μm in diam., some of them fused. Perispore: pale yellow, 0.2–0.6 (0.5) μm thick, strongly attached to the exospore, 1-layered, scabrate.

COMMENTS.—There are variations in spore-sizes and apertural types in this group. In *Sleumer & Vervoorst 2849* (LIL), 32-spored sporangia were found. The spores have different sizes (two normal distributions in the same sample: 59–67 μm and 72–79 μm in equatorial diameter). Monolete (115.2 μm diam) and intermediate (72 μm diam.) spores also were found.

SPECIMENS EXAMINED.—Jujuy, Estación Volcán, *Castillón 24* (LIL), *Castillón 23* (LIL); Salta, Caldera, Potrero del Castillo, *Sleumer & Vervoorst 2849* (LIL); Tucumán, Chicligasta, Bassin Río Cochuna, *Halloy A-137* (LIL); Nevados del Aconquija, *Halloy* s.n. (LIL 559435); Catamarca, Belén, Quebrada del Río Blanco, *Sleumer & Vervoorst 2526* (LIL); Andalgalá, Cuesta de La Negrilla, *Sleumer 2703* (LIL); Andalgalá, Cerro Yutuyaco, *Sleumer 2737* (LIL); Capillitas, *O'Donell 1369* (LIL).

8. **Cheilanthes volcanensis** de la Sota.—Fig. 3E–H.

DESCRIPTION.—Spores: trilete with circular to subcircular outline in polar view; De 45.9–54.0 (50.8) μm; Dp 45–54.0 (50.4) μm. Laesura arms: 19.8–28.8 (23.2) μm long, 2.2–6.3 (3.7) μm high, straight, tenuimarginate or indistinctly marginate, reaching the equator. Exospore: yellow to brown, 1.5–5.2 (2.4) μm thick, 2-layered, smooth. Perispore: yellow, 2.5–3.1 (2.8) μm thick, strongly attached to the exospore, 3-layered; inner layer thin; middle layer well developed composed of threads fused in several levels; outer layer thin; closed structure (Morbelli and Michelena, 1989); cristate. Cristae short, 0.7–2.1 (1.3) μm high; margins supraornate, echinulate; concentrically placed in the distal face. Background sculpture: cristate-perforate.

COMMENTS.—The perispore is cracked and detached as a consequence of the chemical treatment. *Cheilanthes volcanensis* is closely related to *C. marginata.* Most sporophytic features, except the rhizome, are similar in these species, but they can be differentiated easily by their spores.

SPECIMENS EXAMINED.—Tucumán, Chicligasta, Bassin Río Cochuna, *Halloy A-134* (LIL); Cumbre del Malamala, *Lillo 3460* (LIL); Tafí, Ciénaga, Cerro de Las Aguadas, *Lillo 1310* (LIL).

DISCUSSION

From analysis of Tables 2 and 3, which synthesize the data of the species of *Cheilanthes* and *Astrolepis* that grow in northwestern Argentina (Table 2

TABLE 2. Spore characteristics found in Argentinean species of cheilanthoid ferns correlated with species groups recognized by Tryon and Tryon (1982) and Ponce and Morbelli (1989). Data for species indicated with an asterisk (*) are from the first part of this study (Morbelli & Michelena, 1989). Species in parentheses were not studied in this paper (data from other sources).

Species groups and single species	Argentinian Species	Exospore sculpturing	Perispore sculpturing	Perispore structure
C. fraseri	*C. bonariensis*	smooth	rugulate	3-layered
	C. buchtienii		cristate	
	(*C. hypoleuca*)		cristate-reticulate	
C. marginata	*C. marginata**	smooth	rugulate	3-layered
	C. volcanensis		ridged	
	*C. poeppigiana**		cristate	
	(*C. hieronymi*)			
C. microphylla	*C.* cf. *cucullans*	smooth	rugulate	3-layered
	*C. notholaenoides**			
C. micropteris	*C. micropteris**	smooth	cristate	3-layered
	*C. pilosa**		cristate-reticulate	
	*C. pruinata**			
C. myriophylla	*C. myriophylla**	smooth	rugulate	3-layered
C. dichotoma	(*C. dichotoma*)	smooth	cristate	3-layered
	(*C. orbignyana*)		cristate-reticulate	
	(*C. sarmientoi*)			
	(*C. tweediana*)			
C. glauca	(*C. glauca*)	smooth	cristate-reticulate	3-layered
C. obducta	*C. obducta*	smooth	echinate	3-layered
			baculate	
C. squamosa	*C. arequipensis*	verrucate	scabrate	1-layered
	C. squamosa			
A. sinuata	*A. sinuata*	rugulate	folded	1-layered

includes all Argentinean species), the following comments can be made. Seven types of sculpture have been found (Table 2). They are: verrucate, rugulate, equinate-baculate, ridged, cristate, cristate-reticulate, and folded. Three of these types, equinate-baculate, verrucate, and folded, were not cited in the previous study by Morbelli and Michelena (1989). The most frequent types were: rugulate (*C. myriophylla, C. notholaenoides, C. poeppigiana, C. bonariensis, C.* cf. *cucullans*), cristate (*C. micropteris, C. buchtienii, C. volcanensis*), and cristate-reticulate (*C. pilosa, C. pruinata, C. buchtienii*).

Three patterns of sporoderm structure were recognized. 1) Exospore strongly sculptured, verrucate; perispore 0.4–0.8 μm thick, 1-layered, finely sculptured; present in *C. arequipensis* and *C. squamosa.* (Tables 2, 3; Fig. 1).2) Exospore finely sculptured, rugulate; perispore 0.7–1.3 μm thick, 1-layered, folded, finely sculptured; present in *Astrolepis sinuata.* (Tables 2, 3; Fig. 4 E–H). Folds are present only in *Astrolepis.* The perispore is detached from the exospore only in places where the folds rise. Folded perispore differs from rugulate perispore in that the rugae are more compact. 3) Exospore smooth; perispore 2.0–4.5 μm thick, 3-layered, sculptured; inner layer thin, compact, attached to

TABLE 3. Comparative table of some spore features in *Cheilanthes* and *Astrolepis* species of northwestern Argentina. All measurements in μm. Exospore thickness is listed as mean values. Perispore thickness is listed as minimum and maximum values. For all measurements, elements of the sculpturing are excluded. Spores of *C.* cf. *cucullans* were not studied with light microscopy, thus details such as exospore thickness and perispore color were not seen.

Species	Equatorial diameter	Sporoderm pattern	Exospore thickness	Perispore thickness	Perispore color
A. sinuata	53–98	II	2.5	0.7–1.3	Pale brown to yellow
C. arequipensis	49–57	I	2.5	0.4–0.8	Hyaline
C. squamosa	59–67	I	6.4	0.2–0.6	Pale yellow
C. bonariensis	70–80	III	2.2	1.8–4.5	Pale yellow to yellow
C. buchtienii	57–61	III	1.6	1.8–3.6	Brown
C. cf. *cucullans*	46.0–52.5	III	—	0.7–1.2	—
C. obducta	57–61	III	1.3	0.2–0.6	Pale yellow
C. volcanensis	46–54	III	2.4	2.5–3.1	Yellow to brown

the exospore; middle layer composed of fused threads in one to several levels; outer layer thin and sculptured; present in most species of *Cheilanthes.* (Tables 2, 3). In *C.* cf. *cucullans* (0.7–1.2 μm thick) and *C. obducta* (0.2–0.6 μm thick), with a very thin perispore, 3 layers are still clearly distinguishable with LM. The 3-layered perispore of *Cheilanthes,* in spite of its complexity, is weak. It usually is broken into plates, even before any chemical treatment.

According to our observations and those in the literature (Tryon and Tryon, 1982), most cheilanthoids can be grouped by their vegetative features. These groups do not always fit with the associations based on palynological characters. However, spore morphology allows differentiation of closely related species within certain groups with homogenous sporophyte morphology. Such cases include the *C. marginata* group, in which *C. marginata* has ridged spores, *C. poeppigiana* has rugulate spores, and *C. volcanensis* cristate spores. Within the *C. fraseri* group, *C. bonariensis* has rugulate spores and *C. buchtienii* has cristate-reticulate ones, although they are apparently related, according to indument type. This last complex, in which a large number of species are from southern South America, should be revised, because its members present different types of rhizomes, lamina outlines, petiole cross-sections, pinnule margin modifications, and several types of spores. These suggest that the group may be polyphyletic.

In contrast, in other groups of closely related taxa studied previously, such as *C. micropteris* Sw. (Morbelli and Michelena, 1989) and *C. dichotoma* Sw. (Ponce and Morbelli, 1989) all members have cristate-reticulate spores. Minor variations can be documented in perispore thickness and sculpture among these species, such as lumina width and crista height, fusion, and margin characters (smooth or supraornate).

The spores of *C. obducta* are unique. The perispore sculpture is baculate-echinate, with a granulate background and a 3-layered structure. This morphologically isolated species also has other unique sporophytic features, such

as complex hairs composed of several cell types with tongue-and-groove fitting walls, bicellular glandular hairs, vein tips with a T or L form without enlarged tips, and dixylic vascular strands. The two bundles of xylem join at the laminar level. This type of xylem also is found with less frequency in *C. squamosa*. The dixylic structure had been mentioned for *Cheilanthes* by Ogura (1972).

Astrolepis sinuata, C. bonariensis, C. buchtienii, and *C. squamosa* show differences in spore number and size within a sample in most specimens, and in *C. obducta,* between samples. The differences in size are always associated with variations in color and laesura type. Monolete spores often are present in some specimens of *A. sinuata, C. bonariensis,* and in the species that belong to the "verrucate group" (*C. arequipensis* and *C. squamosa*). The reproductive behavior of these taxa should be studied in detail, as there are interesting connections among the spore numbers and types and apogamy, hybridization, ploidy levels, and xerophytism in *Cheilanthes* (Knobloch, 1969; Vida et al. 1971). Further cytological studies of Argentinean cheilanthoid ferns would be very informative.

CONCLUSIONS

The variation in spore morphology of *Cheilanthes* in the studied area is similar to those of other parts of the Americas (cf. Devi et al., 1971; Knobloch, 1969; Knobloch et al., 1970; Tryon and Tryon, 1973), with the exception of *C. obducta,* which does not have similarities with any other American, European, or African species. It has remarkable similarity with those of Australia, particularly to spores of *C. caudata* R. Br., which is endemic to Australia (cf. Chambers and Farrant, 1991; Tryon & Lugardon, 1991, Quirk et al., 1983).

Most *Cheilanthes* species of northwestern Argentina show sporoderm structural pattern III (see Discussion), with a 3-layered perispore. Nevertheless, according to our results (Morbelli et al., 1994) and those reported in the literature (Tryon and Lugardon, 1991), other cheilanthoid ferns, such as some species of *Argyrochosma, Notholaena, Pellaea,* and *Doryopteris,* have a similar structural pattern to *Cheilanthes.*

In spite of the relatively small geographic area studied, we suggest that spore morphology supports some of the taxonomic groups recognized by Tryon and Tryon (1982), such as that of *C. micropteris* and *C. dichotoma,* proposed by Ponce and Morbelli (1989); but not all, such as the *C. marginata* and *C. fraseri* groups. (see Discussion and Table 2).

The "verrucate complex" present in the study area, *C. arequipensis* and *C. squamosa* (with verrucate exospore and 1-layered perispore), is unique not only because of its sporoderm pattern, but because the species have only scales as lamina vestiture (i.e., no hairs) (Tryon and Tryon, 1973, 1982; Tryon and Lugardon, 1991). Other genera of Pteridaceae, such as *Cryptogramma* and *Hemionitis,* have a sporoderm pattern similar to that of the Andean "verrucate complex" (Tryon and Lugardon, 1991). We agree with previous authors that this complex might constitute a distinct genus. A systematic revision of this group is necessary.

Astrolepis sinuata, C. bonariensis, C. obducta, and *C. squamosa* are characterized by 32- and 16-spored sporangia. In *C. buchtienii,* we noted 64-, 32- and 16-spored sporangia. This variation in spore number may reflect an apogamous life cycle in these taxa. In most of these species, there also are different spore sizes and shapes within a sample, probably because of failures in premeiotic mitosis and sporogenesis processes within the sporangia.

Acknowledgments

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Pteris deflexa and its Allies

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ABSTRACT.—The taxonomic status of *Pteris deflexa* and *P. polita* and the nomenclatural status of three other names used in this species group are clarified.

The names *Pteris deflexa* Link, *P. gaudichaudii* J. Agardh, *P. lata* Kaulf. ex Link, *P. polita* Link, and *P. rostrata* Fée all apply to apparently related, free-veined species that occur principally in the Andes mountains or in southeastern Brazil. Over the years, these names have been treated differently in various Floras and indices.

Christensen (1905–1906, pp. 596–605) thought that *P. gaudichaudii* and *P. lata* were likely to be synonyms of *P. deflexa,* that *P. rostrata* was a synonym of *P. gaudichaudii,* and that *P. polita* was a synonym of *P. propinqua.* Based on the examination of material of *P. polita* that they presumed to be authentic, a specimen cultivated in the Botanical Garden at Berlin (*Link* in 1834, HBG—2 sheets), Morton (1967, p. 75) and then Lellinger (1989, p. 179) agreed with Christensen. However, this specimen was collected after the publication of *P. polita,* and so is not a type; furthermore, a satisfactory type for that species exists (see below). Based on an examination of the latter specimen, Tryon and Stolze (1989, p. 75) pointed out that *P. polita* is free-veined, and so cannot be the correct name for *P. propinqua.* The latter is a totally unrelated species with anastomosing veins, uni- or biarcuate veins along the costae between the costules, and also frequent areolae along the segment midribs. Tryon and Stolze placed *P. polita* as a synonym of *P. deflexa.*

Because all of these names were coined long before the type method evolved, the types are sometimes uncertain. That is particularly true for many of Link's species, because over a period of years Link often collected and recollected from plants growing in the garden at Berlin, and he himself did not mark any specimens as types. Link's specimens dated after his publication are not types, of course, but undated specimens and those collected earlier all are potential types. In such cases, taxonomists should examine all of Link's material and choose a suitable lectotype carefully.

Recently, I borrowed and examined the presumable holotype of *P. polita* from Berlin (B, spec. no. 113655; Tryon, photo US). At the lower left is a label with four lines: "Hb. Link/Pteris podophylla Sw./ polita/Litobrochia podophylla." At the right is an annotation on three lines in an old hand, perhaps by Mettenius: "Pteris lata Kaulf./Original exemplar von Pteris polita Lk./Pt. deflexa Lk. var.?" The specimen, apparently a complete frond in two pieces, lacking only some proximal portion of the stipe, can be described as follows: stipe nearly 45 cm long (incomplete), 3 mm wide, stramineous, glabrous; lam-

inae 33 cm long, ca. 25 cm wide at the base, subternate, the basal pinnae bipinnate-pinnatifid, the distal pinnae pinnatifid, the subapical pinnae strongly decurrent at the base from a reduced, basiscopic basal segment; lamina axes stramineous to rufobrunneous abaxially; hairs on the abaxial surface of the costules relatively few, coarse, rather stiff, dark reddish-brown, with distinctly elongate cells.

Based on my examination of the type of *P. polita* and on material and a type photograph of *P. deflexa* in the U. S. National Herbarium, it is clear that these are independent species. Sehnem (1972, pp. 109–114) accepted both species (*P. polita* as *P. gaudichaudii*), which he must have known in the field. He was able to distinguish them by their rhizomes, although I found no differences in their rhizome scales (both have strongly bicolorous rhizome scales with broad, thin, irregular or even ciliate margins). The two can be separated with the following key. The pinnule and pinna dimensions refer to most specimens, but the largest specimens of *P. deflexa* may have dimensions typical of *P. polita,* and the smallest specimens of *P. polita* may have dimensions typical of *P. deflexa.*

Rhizomes creeping, 2–2.5 cm in diam.; subapical pinnae not or slightly adnate to the rachis, but never more than slightly decurrent at the base; hairs of the costae many, lax, pale reddish-tan, with cells generally about as long as wide; plants usually large, the basal pinnules 1–2 cm wide, the suprabasal pinnae 2(3) cm wide (Fig. 2) ..... *P. deflexa*

Rhizomes long-creeping, 0.5 cm in diam.; subapical pinnae strongly adnate to the rachis from a low, triangular lobe to obviously decurrent at the base; hairs of the costae few, stiff, dark reddish-brown, with cells generally at least twice as long as wide; plants usually medium-sized, the basal pinnules (1.5)2–3 cm wide, the suprabasal pinnae 2–3.5 cm wide (Fig. 1) ..... *P. polita*

For the species in the following list that I believe are valid, I have cited a few specimens (all from US) from throughout their range to aid others in identifying their material.

Pteris deflexa Link (Hort. Berol. 2:30. 1833).—TYPE: Cultivated in the garden at Berlin, originally from Brazil, *Link* s.n. (B, not seen; Tryon, photo US; Prado, xerocopy US).

The distal pinnae are pale, narrow, and subequal at the base. A valid species known from Hispaniola (*Ekman H-7854, H-11792*), Puerto Rico (*Britton & Marbler 1064, Sintenis 2018*), Venezuela (*Fendler 102*), Colombia (*Ewan 16504, H. H. Smith 1083*), Ecuador (*Rimbach 313*), Peru (*Killip & Smith 23902, Spruce 4326)* Bolivia (*Buchtien 497, 3541, 5273, Rusby 116*), northern Argentina (*Venturi 111, 1993, 8879, 9070*), Paraguay (*Balansa 2835, Fiebrig 6025*), southeastern Brazil to Est. Minas Gerais (*Dusén 15282, Mexia* 4853, *Rose & Russell 20773, Wacket* [*Ros. Fil. Austrobras. Exs. 121a*]), and Uruguay (*Arechavaleta 68*). Fig. 2.

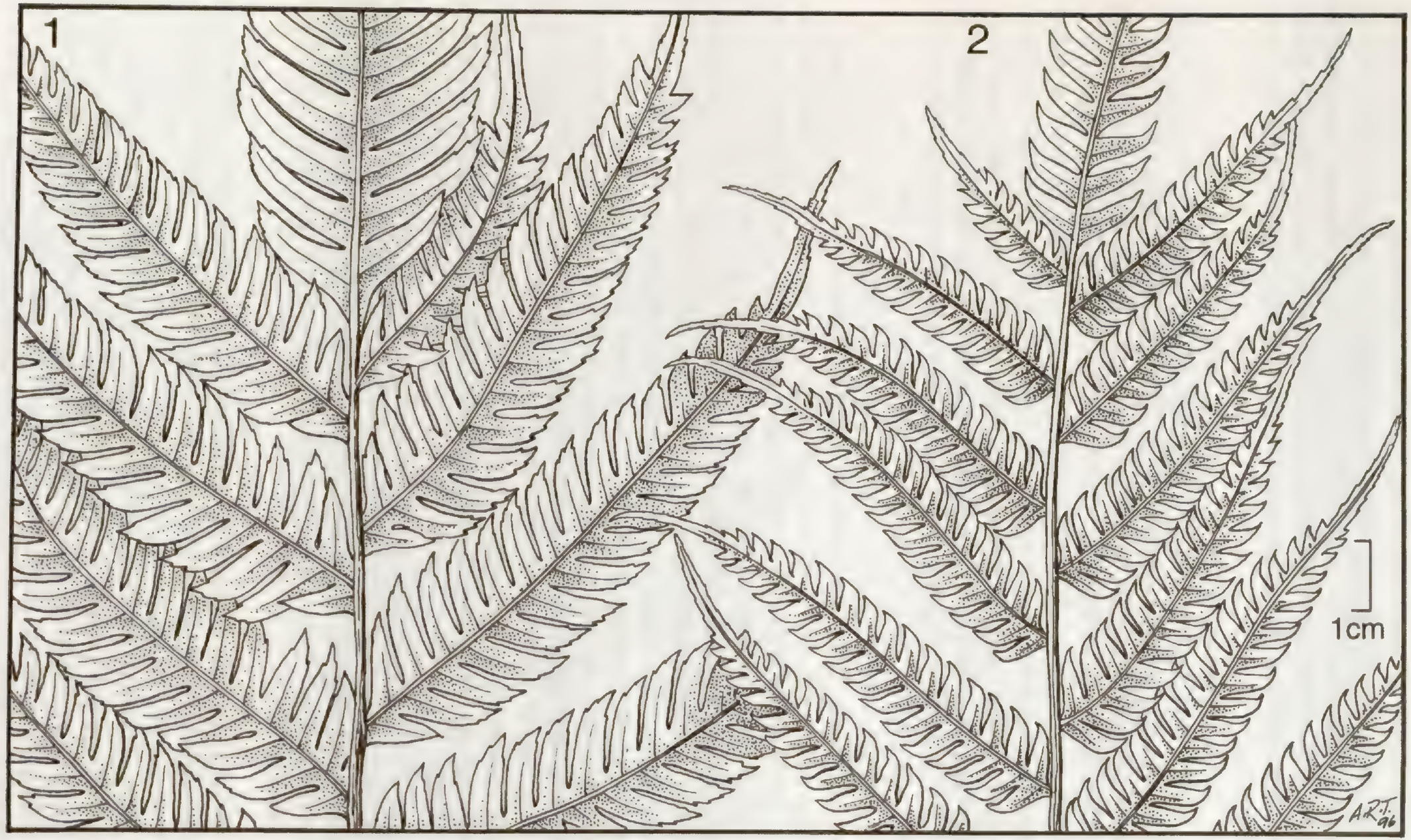

FIG.1. Lamina apex of *Pteris polita* showing adnate distal pinnae with asymmetrical bases (Brazil, *Riedel* s.n., US).
FIG.2. Lamina apex of *P. deflexa* showing non-adnate distal pinnae with symmetrical bases (Brazil, *Mexia 4853*, US).

Pteris gaudichaudii J. Agardh (Recens. Spec. Pter. 42. 1839).—SYNTYPES: In or near Rio de Janeiro, Brazil, *Gaudichaud* s.n. (G, not seen; xerocopy US; isosyntype LU, not seen; Prado xerocopy US) and *Lund* s.n. (AWH, not seen; isosyntype LU, not seen; Prado, xerocopy US).—LECTOTYPE (chosen here): *Lund* s.n. (AWH).

The isosyntype fragment at LU collected by Lund consists only of one or two pinnae and is too fragmentary to be identified with certainty; it may be *P. deflexa*. The isosyntype fragment at LU collected by Gaudichaud matches the presumable holotype of *P. polita* better than that of *P. deflexa* in having pinnae that are darker in color and relatively wider and more asymmetrical at the base than are those of *P. deflexa*. In addition, the xerocopy of the syntype of this collection is of the apical portion of a lamina. It has pinnae similar to the fragment and, more importantly, the subapical pinnae are strongly adnate to the rachis and have a suggestion of a triangular basal basiscopic lobe. A label "TYPUS" was pinned to this sheet sometime in the past. To fix the application of the name *P. gaudichaudii*, I choose this specimen as lectotype. A synonym of *P. polita*.

Pteris lata Kaulf. ex Link (Hort. Berol. 2:28. 1833).—TYPE: Presumably the same as the holotype of *P. polita*. If so, a nomenclatural synonym of *P. polita*, with which the original description more or less agrees.

Pteris polita Link (Hort. Berol. 2:30. 1833).—TYPE: Cultivated in the garden at Berlin, originally from Brazil, *Link* s.n. (B; Tryon, photo US). A valid species known from southeastern Brazil (*Glaziou 435, Rose & Russell 20786, Smith & Brade 2222*) and perhaps Guyana (*Schomburgh*). Fig. 2.

Pteris rostrata Fée (Icon Esp. Nouv. 16, t. 33, f. 1 [Mém. Fam. Foug. 10]. 1865).—TYPE LOCALITY: "Habitat in America aequinoxiali," indicating an Andean origin.

Windisch (1982, p. 60) found two specimens, "Perou," *Spruce* (RB, not seen) and "In monte Campana prope Tarapoto," Peru, *Spruce 4326* (US), that are possible types of *P. rostrata*. Both Fée's figure and the latter specimen match *P. deflexa* better than *P. polita*. A synonym of *P. deflexa*.

Acknowledgments

I am grateful to Jefferson Prado for sending me xerocopies of critical type specimens and his drawings of *P. deflexa* and *P. propinqua*, to Paulo Windisch for his useful suggestions on the manuscript, and to Alice Tangerini for preparing the illustration.

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Leaf Size and Biomass Allocation in *Thelypteris dentata, Woodwardia virginica,* and *Osmunda regalis* in Central Florida

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ABSTRACT.—The sizes of leaves and division of biomass within leaves were examined for separate populations of *Thelypteris dentata, Woodwardia virginica,* and *Osmunda regalis* growing in similar habitats. All three species showed considerable variation in leaf characteristics. *Thelypteris dentata* had the densest population of leaves per m^2, the greatest within-species variation in leaf length and weight, the greatest specific leaf area (area:weight), and the highest ratios of leaf length: mass and leaflet:stalk. *Thelypteris dentata* exhibited leaf characteristics indicative of a stress-tolerant strategy, whereas *W. virginica* and *O. regalis* exhibited a number of the characteristics commonly associated with competitive plants.

Life-history characteristics of seed plants have been used to identify strategies that have evolved to fit environmental characteristics. In the three-strategy, C-S-R model proposed by Grime (1979), plants exist in particular places at particular times as a result of reaching an equilibrium between the intensities of stress, disturbance, and competition. Autecological studies of numerous plant species have allowed plant ecologists to generate a list of morphological, physiological, and life-history attributes associated with the three, main strategies; competitive, stress-tolerant, and ruderal (Grime et al., 1988). One characteristic used to distinguish between these strategies is growth, with the most rapid rates generally found in environments with abundant resources and relatively low disturbance, although plant species may differ widely in their relative rate of biomass production, even when grown as isolated individuals under productive conditions (Garnier, 1991). There are also differences in the growth of individual plants and of plant populations, which may be a mechanism whereby species with more competitive strategies rapidly monopolize resources and suppress the growth and regeneration of neighboring plants (Grime et al., 1988).

A second suite of characteristics used to distinguish plant strategies are those related to the size and composition of supporting and photosynthetic structures. Biomass allocation is typically defined in terms of leaf, stem, and root weight ratios and allocation to leaves and stems is of particular importance (Lambers and Poorter, 1992). Patterns, as well as shifts in allocation patterns, reflect selective pressures and may be a measure of the adaptive significance associated with specific plant structures (Harper, 1977). Some ratios are plastic, even in response to small differences in local conditions (Dong, 1995). Therefore, leaves

may represent a particularly sensitive barometer for assessing plant strategies used to adapt to, and succeed under, existing environmental conditions.

Plant strategies have been proposed for a number of herbs, trees and shrubs, bryophytes, and lichens (Grime et al., 1988) but this type of analysis has not been applied to ferns. The great variety in leaf sizes and textures among ferns has been used to qualify species as more primitive or recently evolved (Gifford and Foster, 1989) and also suggests that leaves may provide excellent models for determining life-history strategies appropriate for different environments. Certainly, the reproductive and physiological differences that distinguish ferns from other plants make such an analysis desirable.

We examined the sizes of leaves of three fern species that have contrasting leaf forms but grow in similar habitats. We determined how biomass was divided within the leaf, focusing on leaflets, which function as photosynthetic units, and leaf stalks, which have both photosynthetic and support functions. Underlying our investigation was the question of whether these species exhibit similar strategies under apparently similar environmental conditions in freshwater, forested habitats.

Methods

A clearly defined, monospecific population of each of the three species was selected in the Ecological Research Area, a 220-ha tract of land located in Hillsborough County, west central Florida (28°05′ N, 82°20′ W) in July 1994. The populations of *Thelypteris dentata* (Forssk.) E. St. John and *Woodwardia virginica* (L.) Sm. were approximately 50 m from each other in a forested wetland dominated by elm (*Ulmus americana* L.) and oak (*Quercus laurifolia* Michx.). The population of *Osmunda regalis* L. was located approximately 1 km away, but in a similar habitat. Nomenclature follows that of Wunderlin (1982).

The density of leaves (number of leaves per square meter) for each species was calculated in three areas of each population. Twenty five leaves, over the size range represented, were collected to establish the relationship between length and weight during leaf growth. Their length was measured within 2 hours after collection and the weight of leaflets and leaf stalks measured after drying at 60°C for 2 days. A curve-fitting procedure (DeltaGraph 3.5) was used to determine the relationship between length and weight.

Ten leaves of *T. dentata* and *W. virginica* and 10 clumps of *O. regalis* were randomly selected by throwing a marker into the population. The leaves were clipped at the surface and placed in a labeled plastic bag. The length and area of each leaf was measured within 2 hours of collection. Each leaf was then separated into leaflets and leaf stalks (petioles), placed in individual, labeled aluminum containers, dried at 60°C for 2 days, and weighed.

Two ratios were calculated, both reflecting relative contributions of energy invested in photosynthetic versus support tissue. Leaf length:mass ratios were calculated using the total leaf length and dry mass, and leaflet:stalk ratios were calculated using the dry mass of leaflets to the dry mass of stalks. The specific leaf area (leaf area/dry weight) was also determined.

TABLE 1. Leaf characteristics (mean and standard deviations with sample size in parentheses) of *Thelypteris dentata, Woodwardia virginica,* and *Osmunda regalis* in west central Florida.

Characteristic	*T. dentata*	*W. virginica*	*O. regalis*
Leaves per m^2	43 ± 16 (3)	18 ± 5 (3)	16 ± 3 (10)
Leaf length (cm)	16.4 ± 12.7 (10)	87.6 ± 27.2 (10)	65.9 ± 19.6 (10)
Dry weight of (g) leaves	0.06 ± 0.06 (10)	2.06 ± 1.21 (10)	2.22 ± 1.07 (10)
Specific leaf area (mm^2mg^{-1})	41.4 (10)	22.4 (10)	

RESULTS

All three species showed considerable variation in leaf characteristics (Table 1). *Thelypteris dentata* had the densest population of leaves, but the leaves were considerably smaller and lighter than those of the two other species. *Thelypteris dentata* also had the greatest within species variation in leaf length and weight. The specific leaf area (area:weight) of *T. dentata* was twice that of *W. virginica.* Leaf area was not measured in *O. regalis,* as the instrument used to measure the areas was unavailable.

The relationship between leaf length and mass for a range of leaf sizes was not linear (Fig. 1), but was most highly correlated with a parabolic curve. Thus, *T. dentata* and *W. virginica* devoted more energy to increasing leaf length (or height) early in their development while continuing to increase in biomass later in their development.

The ratio of leaf length:mass was significantly higher and more variable for *T. dentata* than for the other species (Fig. 2). Leaflet:stalk ratios were more consistent between populations than length:mass ratios and were generally highest for *T. dentata,* which also showed greater variation that the other two species (Fig. 3).

DISCUSSION

The populations of *Thelypteris dentata, Woodwardia virginica,* and *Osmunda regalis* showed inter- and intraspecific variation in leaf characteristics that could be the result of different adaptive strategies. *Thelypteris dentata* had the greatest variation in leaf characteristics, both in terms of being different from the other two species, as well as having high variability within the population. *Thelypteris dentata* is an introduced species in Florida, whereas *W. virginica* and *O. regalis* are native to this area. Some differences in characteristics may be due to this, although we cannot determine whether *T. dentata* is different from the native plants because it is introduced, as we have neither other populations nor other introduced species with which to compare our data.

Although variation in leaf size exists for all three species, our observation that plants invest more biomass in leaf height than leaf mass early in leaf expansion suggests that these ferns grow so as to equalize the height of the canopy within the population. The height of the canopy impacts new growth when shading

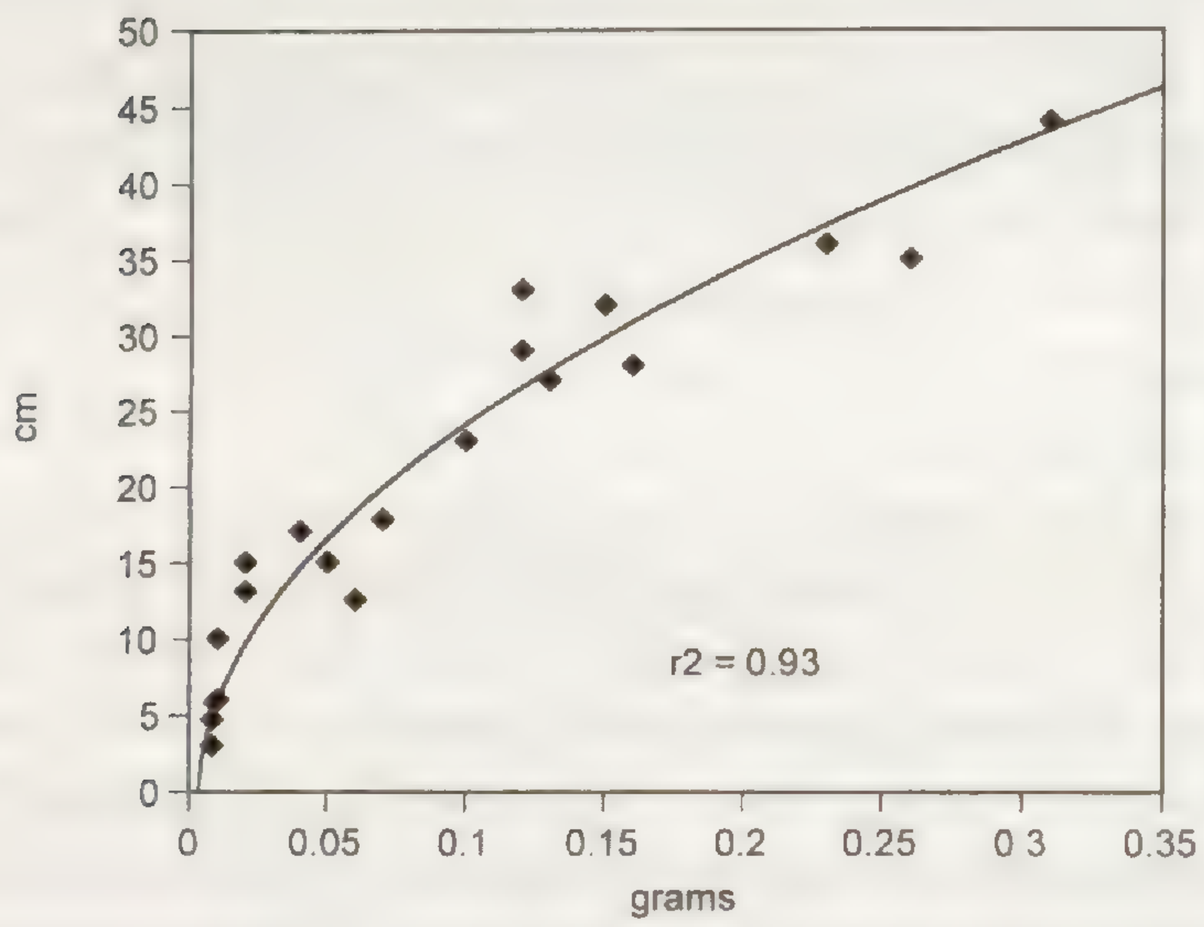

FIG. 1. Relationship between leaf length and leaf mass for (a) *Thelypteris dentata* and (b) *Woodwardia virginica*.

from standing litter (dead leaves) influences the density, size, and growth rate of emerging shoots (Ekstam, 1995). *Thelypteris dentata* does not produce much biomass, and leaves decompose quickly once they become brown and dry. Similarly, *O. regalis* grows in clumps that are slightly more elevated and where drier conditions and increased oxidation promote higher rates of decomposition. However, *W. virginica* has high biomass production, creating dense litter that does not decompose as rapidly and which may affect the size of emerging leaves.

Thelypteris dentata allocates more energy to producing photosynthetic leaf material than supporting leaf material. This is evident not only from the data on ratios of length:weight and leaflets:leaf stalks but also from the qualitatively fragile nature of these leaves. *Thelypteris dentata* also has a higher specific leaf area and may capture more light by displaying leaves horizontally. These leaf adaptations are indicative of a stress-tolerant strategy (Grime et al., 1988). Fur-

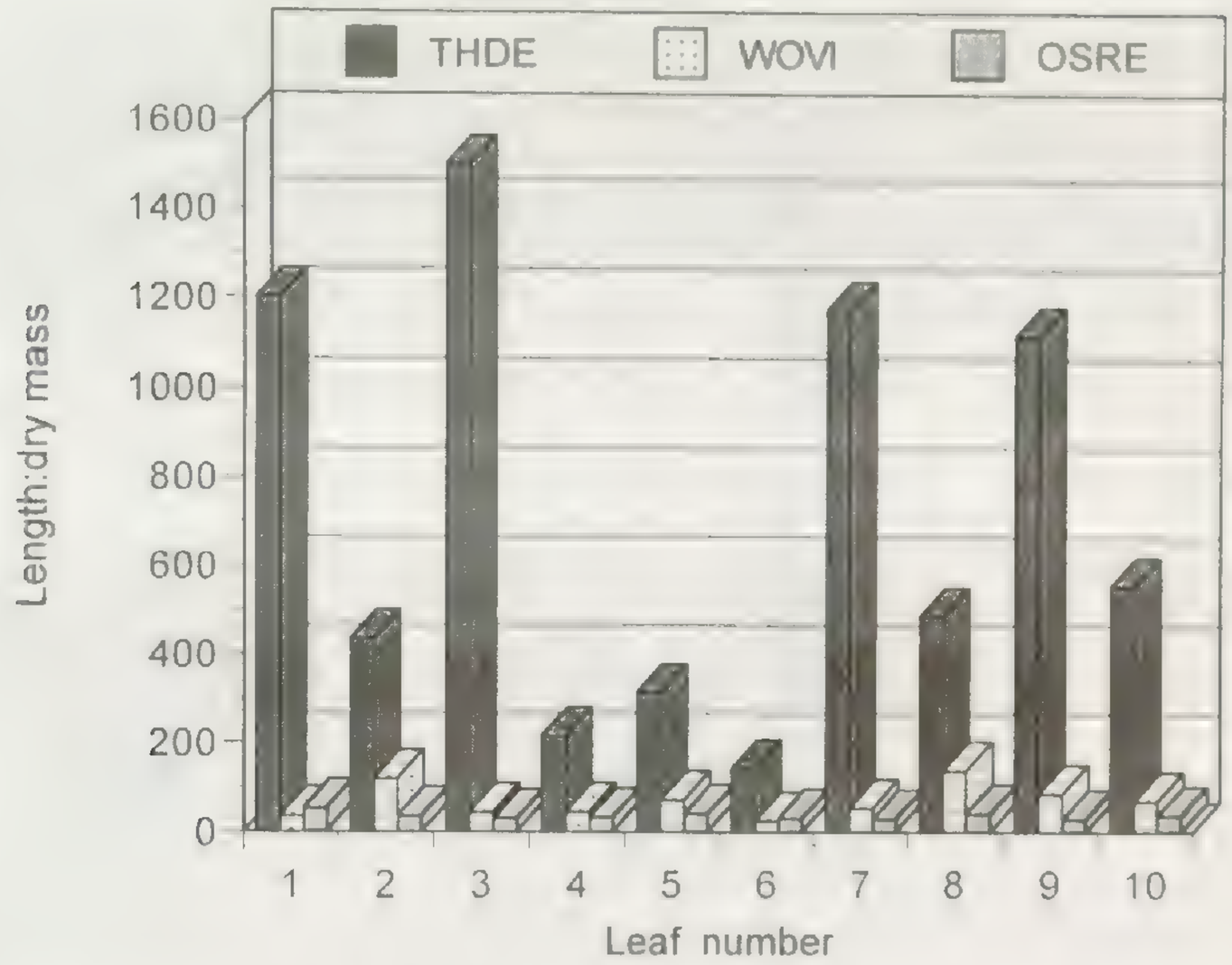

FIG. 2. Ratios of leaf length to leaf biomass for individual leaves of *Thelypteris dentata* (THDE), *Woodwardia virginica* (WOVI), and *Osmunda regalis* (OSRE).

thermore, we have not observed spore production in *T. dentata* in nearly three years of monitoring this population, and lack of investment to reproduction is another measure of a stress-tolerant strategy (Grime et al., 1988).

Woodwardia virginica and *O. regalis* exhibit a number of the characteristics associated with competitive plants including robust and mesophytic leaves, a rapidly ascending monolayer canopy, and high investment in support tissue to provide height growth which increases competitive effectiveness for radiation (Grime et al., 1988). It is not possible to quantify competitive interactions in the absence of shared environmental effects (Mitchell-Olds, 1987) but it is likely that intraspecific competition for light resources occurs in both populations due to the high density of leaves in *W. virginica* and the clumped growth of *O. regalis*.

Competitive plants also tend to have high turn-over rates for leaves since, in a competitive situation, the ability to redistribute resources from old leaves to new and better placed ones may be crucial (Grime 1979). Some of the leaves measured in this study may have been produced at different times during the season, serving different functions as environmental conditions changed over time. The value of a leaf varies with its developmental age (Harper, 1989), and we cannot assume that larger leaves were necessarily older leaves in the case of these ferns.

Although there is a growing body of research related to fern populations (Werth and Cousens, 1990) there continues to be a need for fundamental studies such as these to build our understanding about basic characteristics within this group of plants. In addition to identifying strategies for exotic versus native ferns, future research related to plant strategies should strive to include allocation to reproductive as well as vegetative structures since plant strategies reflect a trade-off between efficiencies in maintaining local populations (growth)

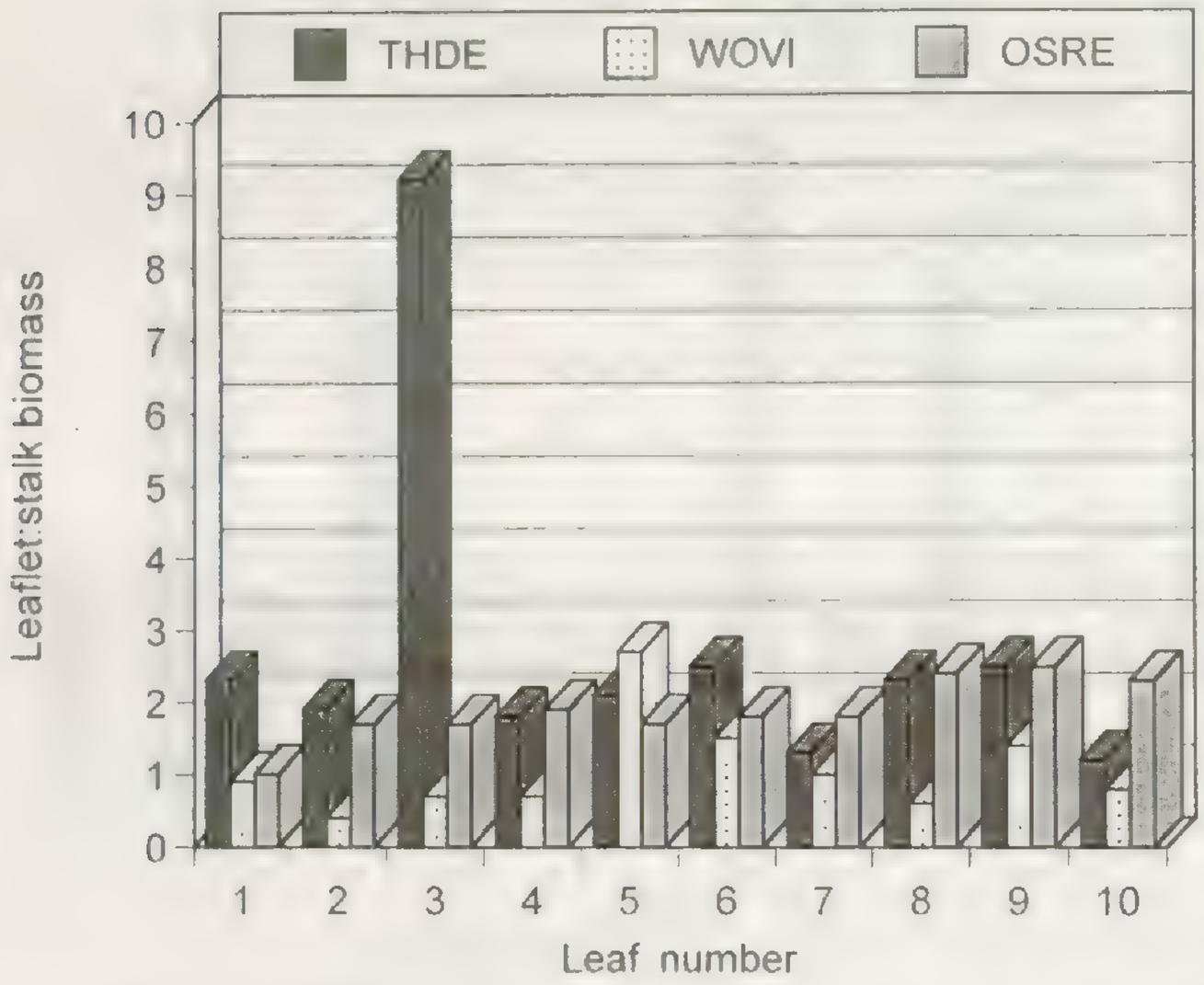

FIG. 3. Ratios of the biomass invested in leaflets (photosynthetic) to that invested in stalk (support) for individual leaves of *Thelypteris dentata* (THDE), *Woodwardia virginica* (WOVI), and *Osmunda regalis* (OSRE).

and expanding populations (reproduction). Our study does not include reproductive allocation, because only *W. virginica* produced sori, although both *T. dentata* and *O. regalis* are capable of producing sori and viable spores. This raises interesting questions about the absolute and relative allocation to reproduction among and between ferns, particularly between species that produce similar fertile and vegetative leaves (where sori are borne on "normal" leaves) versus those that produce modified fertile leaves resembling stalks bearing sori.

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Comparative Gametophyte Morphology of Selected Species of the Family Polypodiaceae

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ABSTRACT.—Gametophyte morphology and growth habit of seven epiphytic species of six genera in the Polypodiaceae are described. In addition to the *Drynaria*-type of early development, depicted previously in most of this family and expressed in all species observed, the *Ceratopteris*-type, *Adiantum*-type, and *Aspidium*-type also occurred in some of the species observed. All species observed were long-lived, clone-forming, and without gemmae. The long-lived, clone-forming habit increases the space and time occupied by gametophytes, and thus enhances the probability of interaction with other gametophytes and the probability of intergametophytic mating in epiphytic habitats.

Gametophyte morphologies vary greatly beyond the standard heart shapes usually depicted in textbooks (Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973). These variations include the ability to grow indeterminately and to branch, so that perennial gametophyte clones of considerable size may be produced. In some families, gametophytes also have the ability to reproduce themselves vegetatively by dispersable gemmae (Farrar, 1993a, b; Raine, 1994; Dassler, 1995). Gemma production and/or clonal growth have been observed primarily in epiphytic species.

Although most of the variation in gametophyte morphology is among epiphytic species, most of the information known on reproductive biology is from research on terrestrial ferns. Epiphytic ferns exist in an environment very different from that of terrestrial species. The gametophytes of tropical epiphytic species are often found within dense bryophyte mats (Dassler, 1995). In such habitats, interaction between gametophytes via chemicals (antheridiogen) and sperm transfer may be significantly hindered relative to gametophytes of terrestrial ferns on smooth surfaces (Dassler, 1995). Gametophyte persistence and expansion by clone formation may help to overcome these difficulties and promote reproduction in the aerial habitat. In order to assess the significance of morphological diversity to fern reproduction in different habitats, further knowledge of the morphology of gametophytes in epiphytic species is needed.

Most species of Polypodiaceae are epiphytic (Lellinger, 1985). We observed the morphology of the gametophytes of seven epiphytic species from this family. Results are interpreted with respect to modes and mechanisms of the species' reproductive biology.

MATERIALS AND METHODS

The species observed are listed in Table 1. Spores obtained from fertile fronds (Chiou and Farrar, 1994) were stored in a refrigerator at about 5°C.

TABLE 1. Polypodiaceae species studied, their distribution, and collection source. All vouchers are at ISC.

Species	Distribution	Collection
Campyloneurum angustifolium (Sw.) Fée	Florida, Mexico, Central and South America	ISU Greenhouse, source unknown
Campyloneurum phyllitidis (L.) C. Presl	Florida, Mexico, West Indies, Central and South America	ISU Greenhouse, source unknown
Lepisorus thunbergianus (Kaulf.) Ching	China, Taiwan, Japan, Philippines, Hawaii	Hawaii, *Farrar 92-8-16-4*
Microgramma heterophylla (L.) Wherry	Florida, West Indies	ISU Greenhouse, source unknown
Phlebodium aureum (L.) J. Sm.	Florida, Mexico, West Indies, Central and South America	Florida, *Chiou 14342*
Phymatosorus scolopendria (Burm.) Pic. Serm.	Ryukyus, Taiwan, Hainan, Malaya, Hawaii	Hawaii, *Farrar 92-8-20-1*
Polypodium pellucidum Kaulf.	Hawaii (endemic)	Hawaii, *Farrar 92-8-19-3*

Voucher specimens were deposited in the Ada Hayden Herbarium (ISC) of the Department of Botany at Iowa State University.

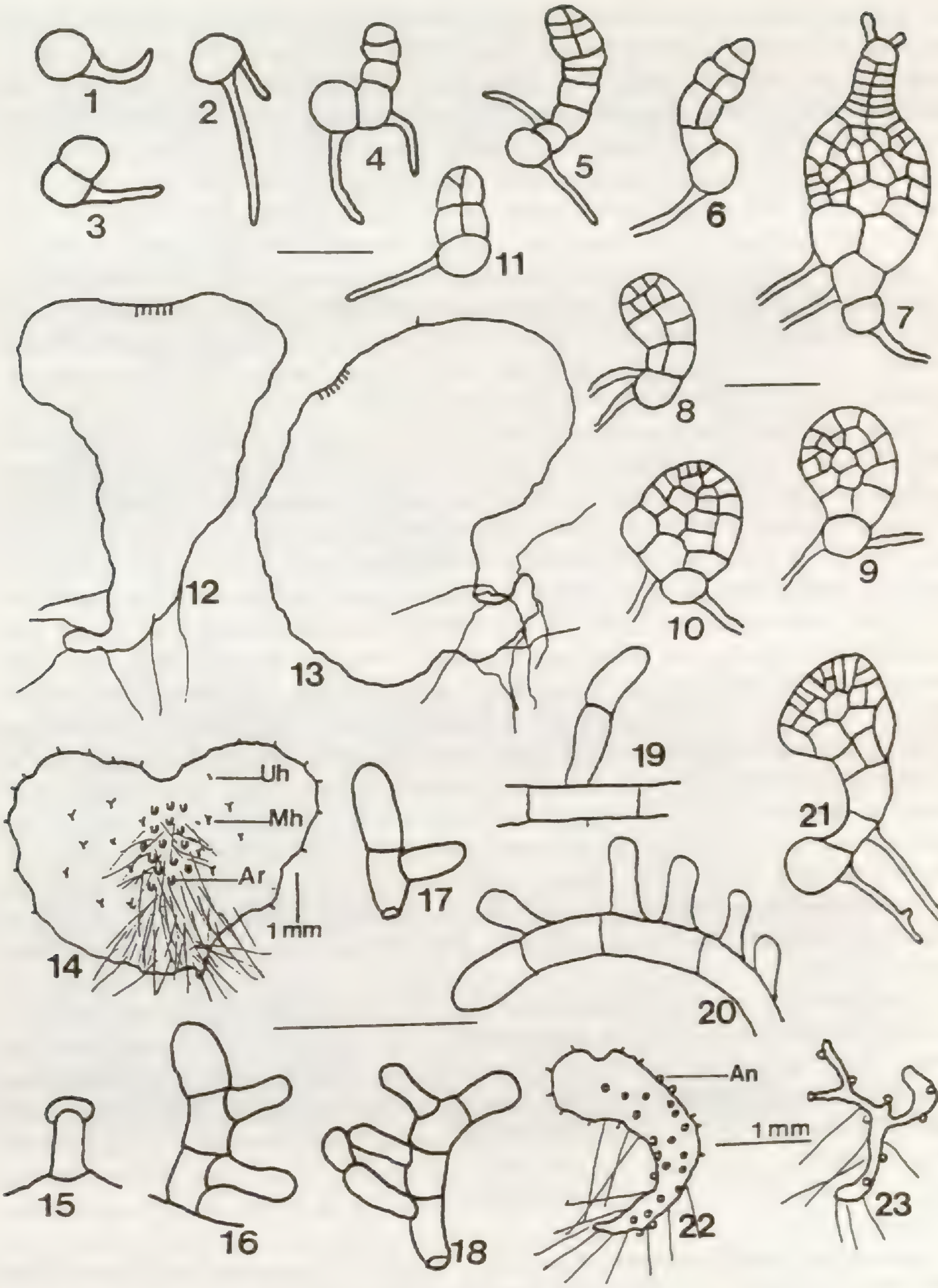
Spores were sown on 1% agar-solidified media that contained Bold's macronutrients (Bold, 1957), Nitsch's micronutrients (Nitsch, 1951), and a trace of ferric chloride (Farrar, 1974). Cultures were maintained under continuous, white fluorescent illumination of 2000–3000 lux. Temperature was maintained at 20–24°C.

For microscopic observation, gametophytes were removed from culture and mounted in water. Drawings were made with the aid of a drawing tube. Photographs were taken through compound and dissecting microscopes or a 55 mm macrolens.

RESULTS

In multispore cultures, spores divided first into a basal cell that remained inside the spore wall and a rhizoidal cell that elongated to form a slender, nonseptate rhizoid (Fig. 1). The basal cell underwent a second division (Fig. 3), resulting in a protonemal cell that subsequently produced a filament by serial transverse divisions (Fig. 4). Occasionally, a second rhizoid appeared before the protonemal cell in *Campyloneurum phyllitidis* and *Polypodium pellucidum* (Fig. 2). The lengths of filaments varied from two to eight cells. Additional rhizoids also emerged from filament cells below the apical cell of the protonema. Filament growth was terminated by formation of a division parallel to the axis of the filament (longitudinal division), usually first in the apical cell, but occasionally beginning in other cells of the filament (Figs. 5, 6). Sometimes all the filament cells except the basal cell eventually divided longitudinally (Fig. 8). Subsequent divisions and expansion of filament cells formed a broad spathulate prothallus (Fig. 9).

The development of the meristem varied among species. In most plants of



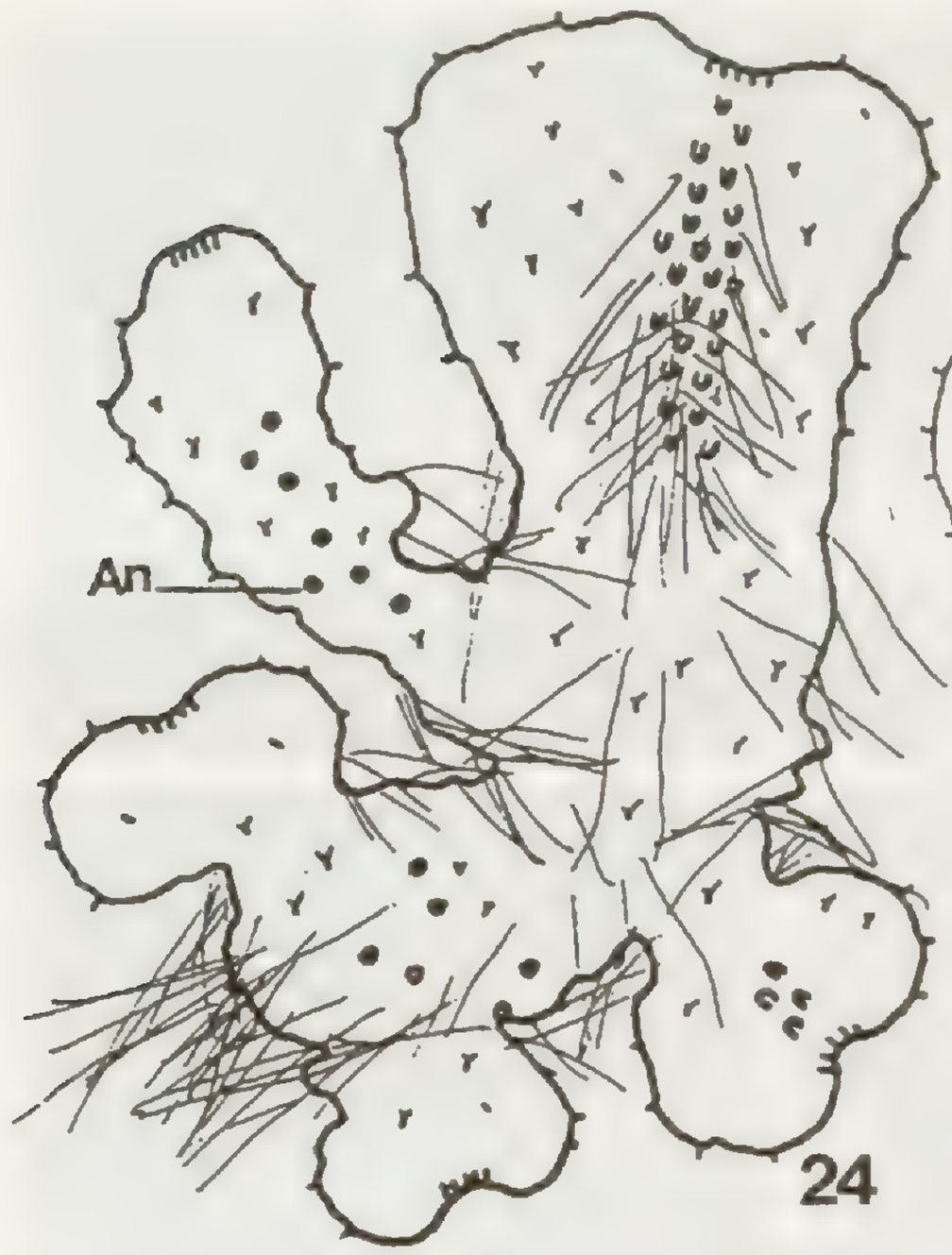
FIGS. 1–23. Development and morphology of Polypodiaceae gametophytes. 1–4) *Campyloneurum phyllitidis*. 5–10) *Microgramma heterophylla*. 11) *Polypodium pellucidum*. 12–18) *Campyloneurum phyllitidis*. 19–21) *Phymatosorus scolopendria*. 22–23) *Polypodium pellucidum*. An = antheridium. Ar = archegonium. Mh =multicellular hair. Uh = unicellular hair. Scale bar = 0.1 mm unless otherwise indicated.

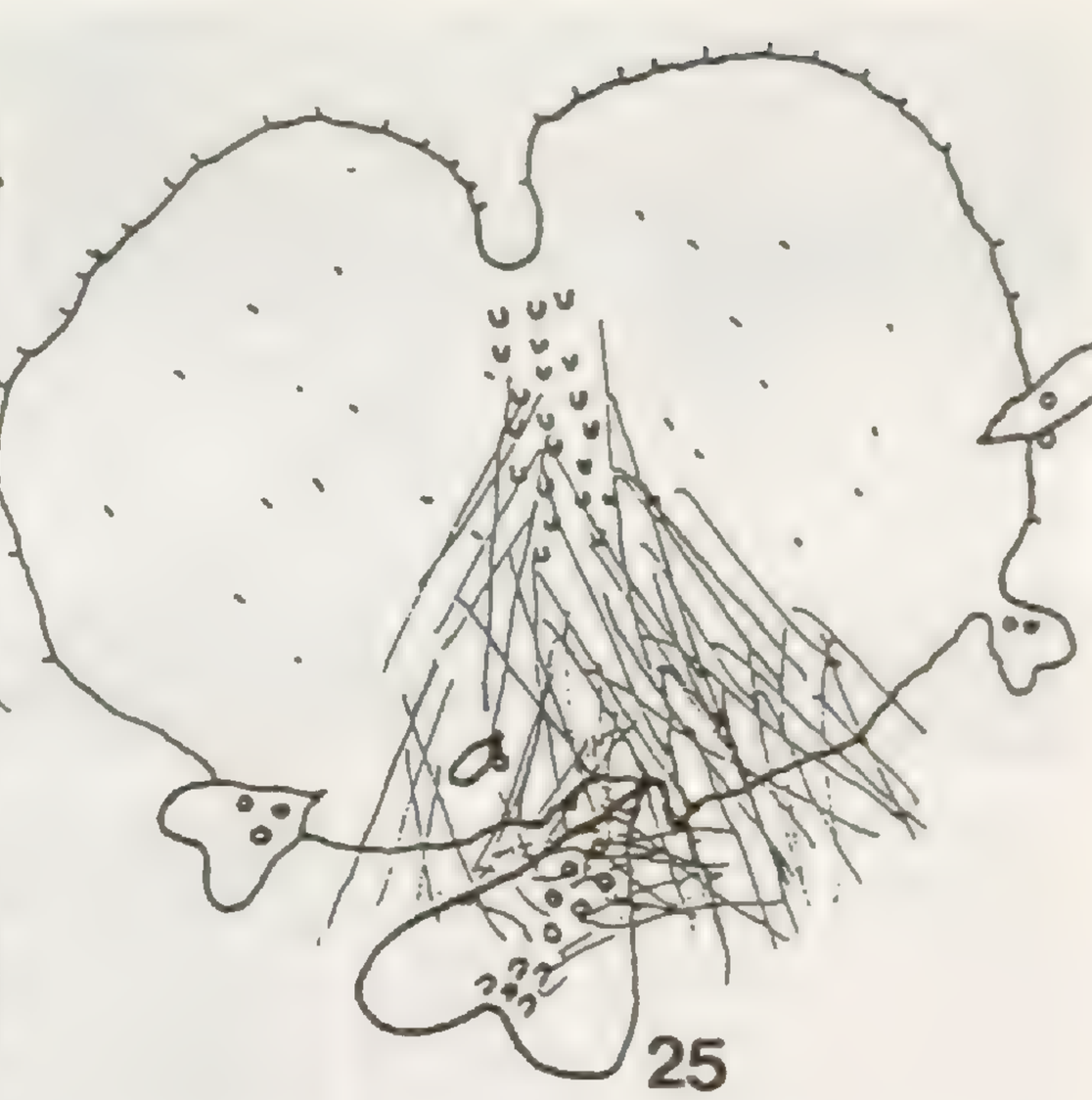
all species examined, an obconical meristematic cell was built at the anterior margin of the spatulate thallus by two oblique divisions (Figs. 10, 21). In *P. pellucidum,* the obconical meristematic cell sometimes formed early in the biseriate stage of development (Fig. 11). In all species, a pluricellular meristem eventually replaced the single meristematic cell (Fig. 12). In *Campyloneurum phyllitidis, Phlebodium aureum,* and *Polypodium pellucidum,* the pluricellular meristem was sometimes initiated on the side of a spatulate prothallus (Fig. 13). In *Microgramma heterophylla,* a lateral or sublateral meristem developed if the apical cell of the filament produced a trichome (Fig. 7). Eventually, a symmetrical (or nearly) cordate gametophyte formed no matter which type of meristem developed initially (Figs. 12, 14). In *Campyloneurum phyllitidis* and *Microgramma heterophylla,* when the width of the thallus was about 3–4 mm, gametophytes elongated and became strap-like.

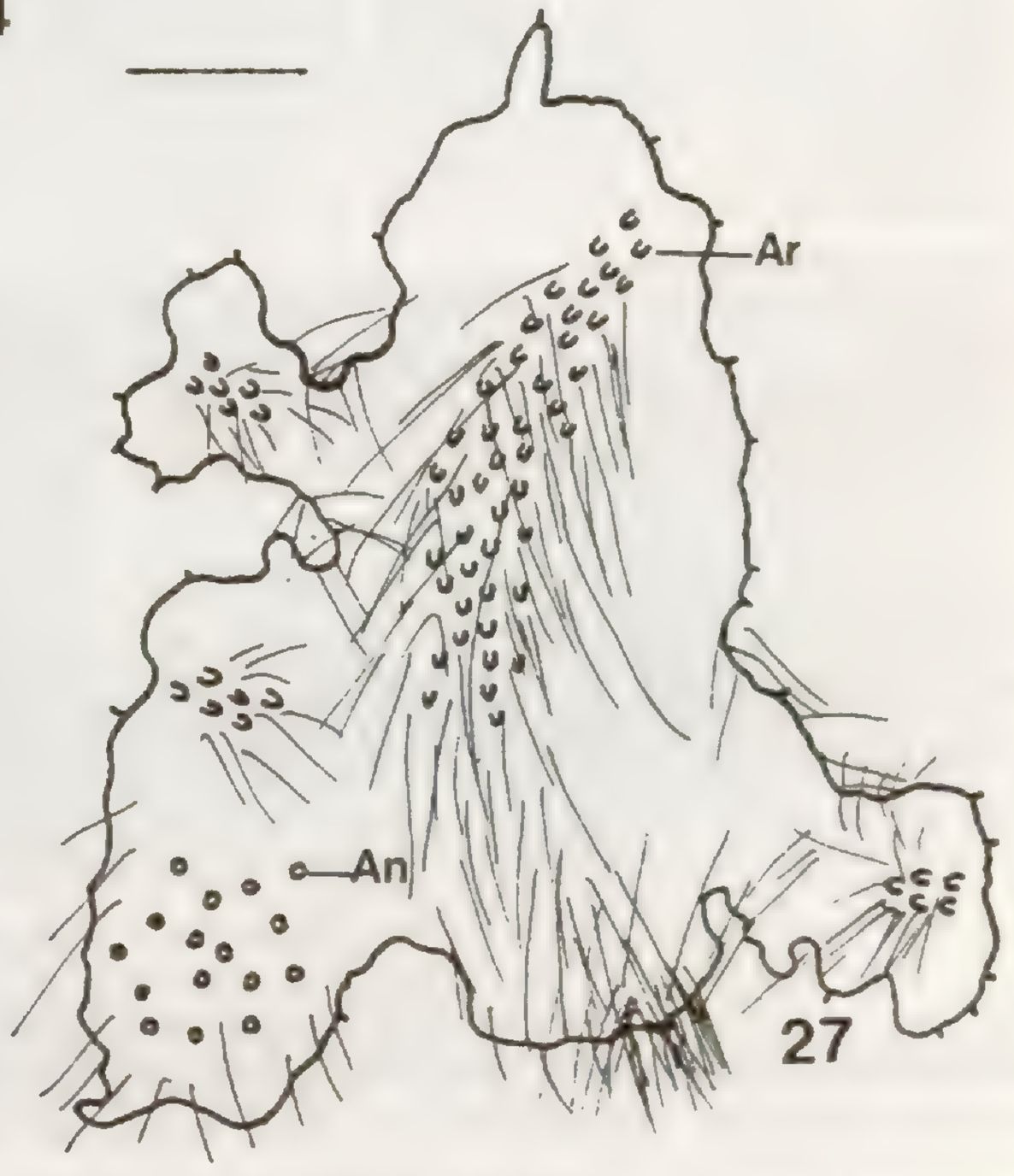
After attaining late spatulate or early cordate stage, gametophytes produced hairs, most of which were unicellular, papillate, and secretory. These first appeared on the margins (Figs. 7, 13), then on both dorsal and ventral sides of wings and midribs (Figs. 14). A secretion often capped the glandular hairs (Fig. 15). On older gametophytes (usually those which had developed a thickened midrib), some hairs became larger and some branched or nonbranched multicellular hairs appeared (Figs. 15–20), except for *Lepisorus thunbergianus* and *Phlebodium aureum* in which no multicellular hairs formed. Usually, a gametophyte cell produced only one hair, but sometimes two hairs were present on one cell, e.g., in *Microgramma heterophylla* and *Polypodium pellucidum* (Fig. 7). The distribution of hair type varied in some species. For example, in *Campyloneurum phyllitidis* and *Microgramma heterophylla,* unicellular hairs usually appeared on the margin and only occasionally on the surface, especially on older gametophytes, whereas multicellular hairs frequently occurred on both dorsal and ventral sides of wings and midribs, but rarely on the margin of the gametophyte (Figs. 14). In *Lepisorus thunbergianus,* the number of the marginal hairs varied greatly, and surface hairs were absent in some gametophytes.

In addition to growing from the margin, rhizoids also occurred on both dorsal and ventral sides of the midrib as well as the wings, but those on the wings were fewer than those on the midrib or on the margin. Rhizoids sometimes emerged from archegonia, e.g., in *Lepisorus thunbergianus* and *Polypodium pellucidum.* Clusters of marginal rhizoids sometimes appeared on *Lepisorus thunbergianus,* especially on relatively elongated strape-like gametophytes (Fig. 26). Branched rhizoids occasionally occurred in *Phymatosorus scolopendria* (Fig. 21).

Antheridia usually appeared on the posterior of gametophyte plants, but sometimes formed near the apical meristem if a midrib had not formed, or on the margin, especially on small irregular-shaped gametophytes (Figs. 22, 23) that usually occurred later in the culture period or in areas of high gametophyte density. Archegonia always were distributed along the midrib behind the meristem (Fig. 14, 24–27). In *Lepisorus thunbergianus,* archegonia were sometimes located along a discontinuous midrib (Fig. 28). In *Lepisorus thun-*



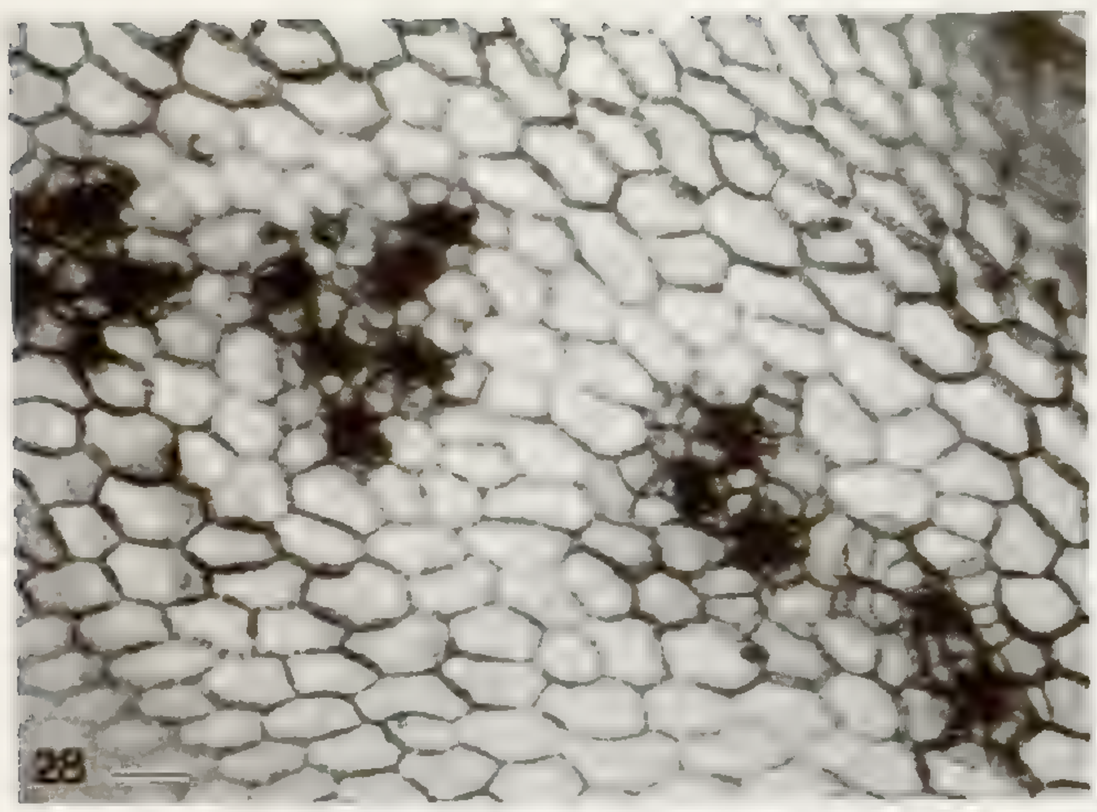


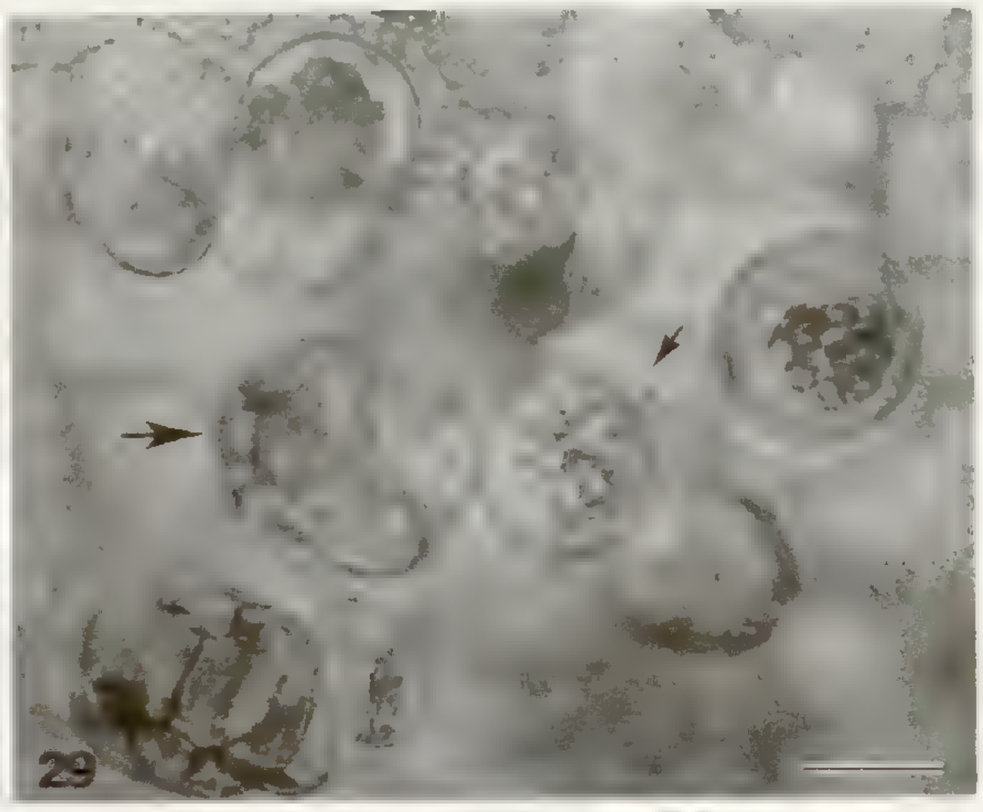


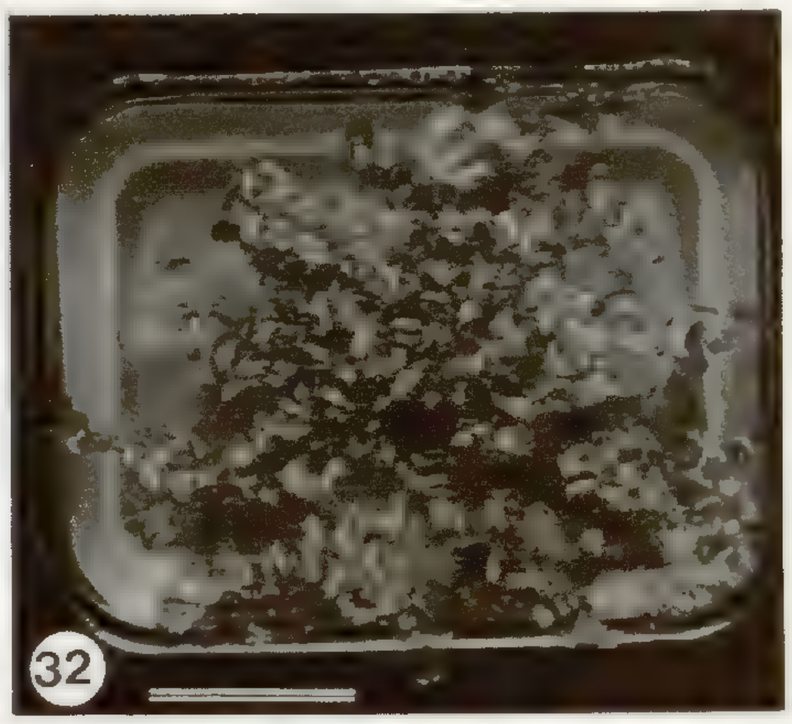
FIGS. 24–27. Clone formation by Polypodiaceae gametophytes. 24) *Campyloneurum phyllitidis*, four months old. 25) *Phlebodium aureum*, two months old. 26) *Lepisorus thunbergianus*, six months old. 27) *Microgramma heterophylla*, four months old. An = antheridium. Ar = archegonium. Scale bar = 1 mm.

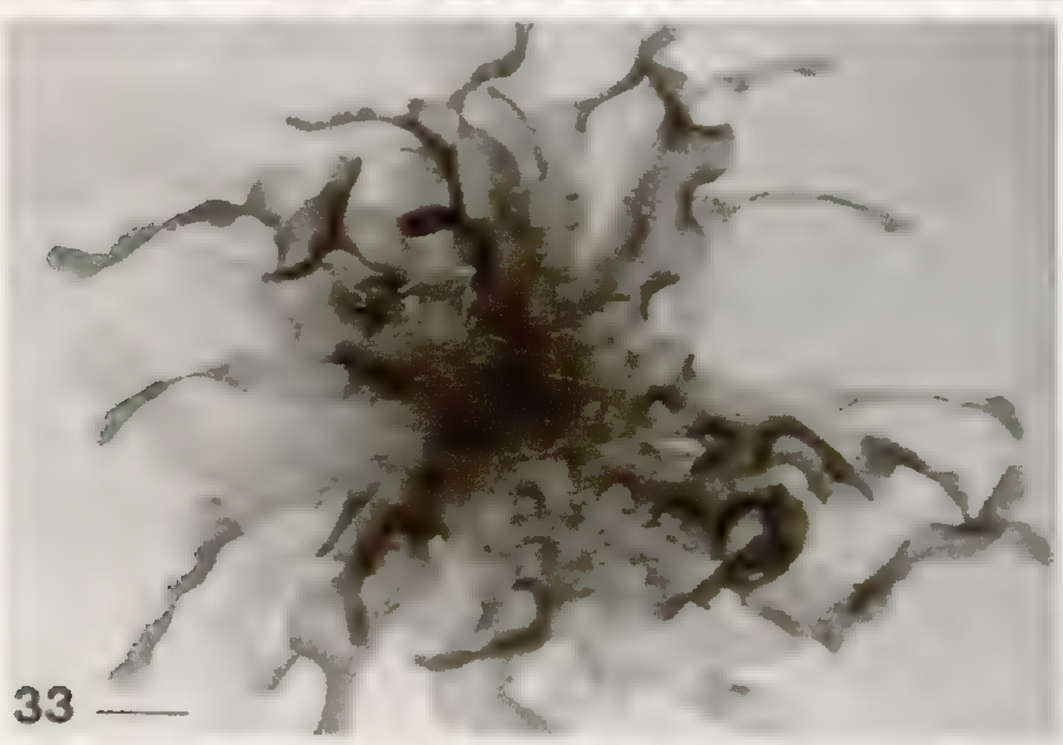
bergianus and *Phlebodium aureum*, antheridia became mixed with archegonia on the older gametophytes (Fig. 29).

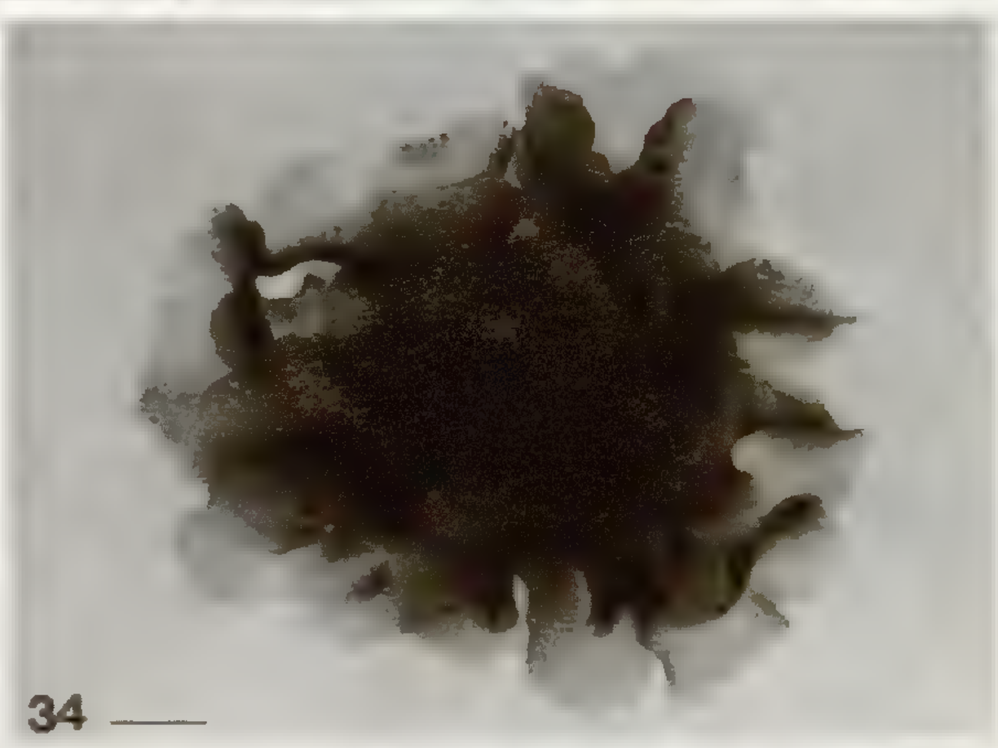
When gametophytes reached their maximum width or nearly so, branching occurred. Two kinds of branching were observed: 1) a broad segment of mar-











FIGS. 28–34. Morphology and clone formation in Polypodiaceae gametophytes. 28–31) Gametophyte morphology. 28, 29) *Lepisorus thunbergianus;* 28) archegonia on discontinuous midribs; 29) antheridia (small arrow) mixed with archegonia (large arrow); 30) proliferations on the margin of *Microgramma heterophylla*; 31) proliferations on the surface of *Phlebodium aureum*. 32–34) Clones formed from single spores. 32, 33) *Microgramma heterophylla*; 32) two years old; 33) eight months old. 34) *Campyloneurum angustifolium,* six month old. Scale bar = 0.1 mm for Figs. 28–31; 1 cm for Fig. 32, 1 mm for Figs. 33, 34.

ginal cells became a pluricellular meristem that produced a branch (Figs. 24, 27); 2) one or a few cells produced a proliferation similar to a prothallus that elongated and expanded to some degree, then developed a pluricellular meristem (Fig. 25). The second type was common to gametophytes of all species studied, whereas the first type was seen only in *Campyloneurum angustifolium, Campyloneurum phyllitidis, Lepisorus thunbergianus,* and *Microgramma heterophylla* (Table 2). In *Lepisorus thunbergianus,* the principal meristem also

TABLE 2. Summary of gametophyte habitat, morphology, and growth habits in seven species of Polypodiaceae. Abbreviations—Habitat: E = epiphytic, H = hemiepiphytic, R = rupestral, T = terrestrial. Development type: Ad = *Adiantum*-type, As = *Aspidium*-type, C = *Ceratopteris*-type, D = *Drynaria*-type (Nayar and Kaur, 1971). Mature form: C = cordate-thalloid, S = strape-like (Nayar and Kaur, 1971). CL = clone-formation. Branch type: 1 = from a broad segment of marginal cells, 2 = from one or a few cells of the margin or surface.

Species	Habitat	Development type	Mature form	Branch type	Longevity
Campyloneurum angustifolium	E, R	D	C, CL	1, 2	Long-lived
Campyloneurum phyllitidis	E, R	C, D	C, S, CL	1, 2	Long-lived
Lepisorus thunbergianus	E, R	D	C, S, CL	1, 2	Long-lived
Microgramma heterophylla	E, R	As, D	C, S, CL	1, 2	Long-lived
Phlebodium aureum	E, R, T	C, D	C, CL	2	Long-lived
Phymatosorus scolopendria	E, H, R, T	D	C, CL	2	Long-lived
Polypodium pellucidum	E, R, T	Ad, C, D	C, CL	2	Long-lived

divided to form dichotomous branches (Fig. 26). Branches of the second type developed either from the margin (Fig. 30) or from the surface (Fig. 31) of gametophytes. Multiple branching from a single original gametophyte eventually yielded a mat of of overlapping thalli (Figs. 32–34). New proliferations subsequently produced gametangia. Gametophyte clones continued growing and after two years had produced multiple sporophytes from different thalli of the clone. Clonal expansion continued after sporophyte initiation through growth and branching of non-sporophyte-producing apices (Fig. 35, 36).

DISCUSSION

The early gametophyte development of Polypodiaceae has been classified previously as primarily *Drynaria* type, rarely *Kaulinia*-type or *Aspidium*-type (Nayar and Kaur, 1971). In this study, the *Drynaria*-type appeared in every species. An additional type (*Ceratopteris*-type) occasionally occurred in *Campyloneurum phyllitidis, Phlebodium aureum,* and *Polypodium pellucidum*. In *Polypodium pellucidum,* the *Adiantum*-type and in *Microgramma heterophylla,* the *Aspidium*-type also appeared (Table 2). Thus, early development seems to be somewhat plastic and perhaps of limited usefulness as a character for systematic purposes.

Gametophytes of most, perhaps all, fern species are capable of regenerating new prothalli from older ones (Atkinson and Stokey, 1964; Nayar and Kaur, 1971). Likewise, all gametophytes have potential for continued growth of the original meristem in the absence of sporophyte production. However, a significant quantitative difference exists in species' expression of these capabilities. Gametophytes of terrestrial species, when grown in sufficient light and space, generally remain as single cordate individuals over usual periods of laboratory culture (2–6 months). This is also the case for gametophytes of terrestrial species we have observed in the wild for up to one year (Peck et al., 1990). In contrast, gametophytes of epiphytic Vittariaceae and Hymenophyl-

FIGS. 35 and 36. Several sporophytes (arrows) derived from gametophyte clones produced from single spores. 35) *Campyloneurum phyllitidis,* 2.5 year old. 36) *Phymatosorus scolopendria,* 1.5 year old. Scale bar = 0.5 mm for Fig. 35; 1 cm. for Fig. 36.

laceae branch at an early age in culture (<1 month) as part of normal maturation, and wild gametophytes of these families and of Grammitidaceae are nearly always branched (Farrar, 1993a, b; Dassler, 1995). This seemingly programmed development of branching also characterizes all the Polypodiaceae species in this study.

Gametophytes of many epiphytic species (especially Vittariaceae and Hymenophyllaceae) also differ from those of terrestrial species in that cells derived from the apical meristem expand primarily along their longitudinal axis, creating an elongated strap- or ribbon-like thallus, whereas gametophytes of most terrestrial species expand primarily in width, creating the familiar butterfly- or heart-shaped thallus. Both types of expansion are displayed among the species of Polypodiaceae. Mature gametophytes of *Christiopteris, Colysis, Dendroglossa, Kaulinia, Leptochilus,* and *Paraleptochilus* have been described as ribbon-like with a broad, rounded meristematic apex, those of *Selligua* and some species of *Lepisorus* as elongated and strap-like with an apical notch meristem, and most Polypodiaceae species as cordate-thalloid with an apical notch meristem (Nayar and Kaur, 1971). In our study, gametophytes of *Campyloneurum phyllitidis, Lepisorus thunbergianus* and *Microgramma heterophylla* exhibited strap-like growth as mature plants, whereas individual thalli of *Campyloneurum angustifolium, Phlebodium aureum, Phymatosorus scolopendria,* and *Polypodium pellucidum* remained cordate, except when they grew in very high density or under very low light intensity where they became slender and strap-like. Both types of growth permitted multiple and continuous production of sporophytes from different thalli of clones derived from a single spore. Thus, although morphology of the mature thallus varies within the Polypodiaceae, persistent growth and clone formation may be characteristic of the family.

Starch-gel enzyme electrophoresis studies by many researchers indicate that most terrestrial sporophytes of homosporous ferns are produced through outcrossing (e.g., Soltis and Soltis, 1992). Assuming increased difficulty of sperm

transfer in the bryophyte dominated reproductive habitats of epiphytic ferns, one might expect a high degree of selfing in epiphytic species. To the contrary, enzyme electrophoresis studies have indicated that epiphytic ferns also tend to be outcrossing (Masuyama et al., 1987; Ranker, 1992; Haufler, 1995). Could the gametophyte morphology of epiphytic ferns be an adaptation promoting outcrossing? Long life and clone formation have been hypothesized to be of adaptive value both in competing with bryophytes in the epiphytic habitat and in increasing the opportunity for gametophyte interaction (Dassler, 1995). Dassler stated that this growth habit "... allows a fern gametophyte to physically migrate to a microsite favorable for growth and reproduction, as well as to survive until favorable microsite conditions occur, and to persist until the arrival of a second migrant", thus facilitating outcrossing. The perennial clone-forming habit also assures continued presence of gametes due to production of gametangia on new proliferations. If we consider antheridiogen function, the promotion of gametophyte interaction by clone-formation and long-life is even more significant, and includes the potential for interaction with previously buried spores (Haufler and Welling, 1994; Chiou, 1996).

Numerous descriptions of gametophyte morphology by A. G. Stokey, L. R. Atkinson, B. K. Nayar and many others (Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973) have characterized and differentiated fern taxa according to spore germination, early development type, mature form, trichome type, gametangium type and position, etc., and combinations of these characters. Although these studies have established the potential contribution of gametophyte characters to fern systematics, little attention has heretofore been given to the functional basis of gametophyte form. The association of certain gametophyte characteristics, i.e. those contributing to the formation of long-lived clones, with certain habitats, i.e. tropical epiphytic, calls for careful examination of gametophyte characters with consideration of possible parallel evolution of morphological characters among taxa.

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Establishment and Maintenance of Aseptic Culture of *Trichomanes speciosum* Gametophytes from Gemmae

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ABSTRACT.—A technique is described for surface sterilization of gemmae of *Trichomanes speciosum*. The subsequent aseptic growth of gametophytes in artificial media under different temperature and light regimes is described. The utility of the technique as an alternative to surface sterilization of spores for the inception of gametophyte cultures of gemmiferous ferns and bryophytes is demonstrated.

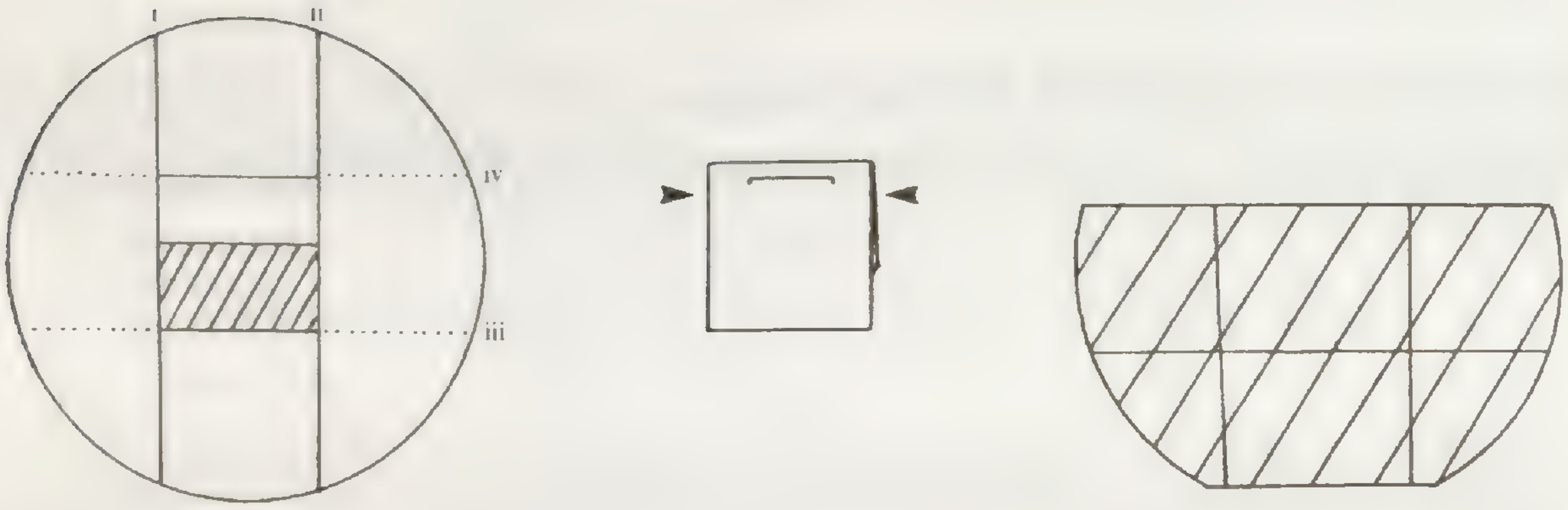
Aseptic culture of fern gametophytes is routinely initiated from surface-sterilized spores (e.g., Dyer, 1979). There are many reasons for generating such cultures, but most concern the elucidation of substrate requirements or documentation of growth and reproductive behavior in defined conditions. Gametophytes of the filmy ferns (Hymenophyllacaeae) have been cultured from spores by several workers, largely with the aim of observing their subsequent ontogeny (e.g., Stokey, 1940; Stone, 1958; Yoroi, 1972). These workers reported successful culture, but admitted to significant losses through contamination and slow germination and growth.

The Killarney fern, *Trichomanes speciosum* Willd., is one of the rarest ferns in Europe, and a licence is required for the collection of any material (Perring and Farrell, 1983). Sporophytes produce chlorophyllous spores (Stokey, 1940) very infrequently in Britain (e.g., Sheffield, 1994). The spores germinate and grow extremely slowly (Yoroi, 1972). Collection and surface-sterilization of spores of this species are therefore fraught with problems.

One way to avoid the problems caused by using spores is to use gametophytic tissue as starter material for culture. *Trichomanes speciosum* gametophytes occupy many more sites than sporophytes in Europe (e.g., Vogel et al., 1993) and gemmae are produced at most sites (Rumsey, 1994). Gemmae germinate and produce gametophytes more rapidly than do spores (Stone, 1965). The aim of this investigation was therefore to test *Trichomanes speciosum* gemmae for their suitability as material with which to establish aseptic cultures of this endangered species. Achievement of this aim necessarily involved testing the ability of various culture conditions and defined artificial media to support germination of gemmae and the subsequent growth of the filamentous gametophytes of *Trichomanes speciosum*.

MATERIALS AND METHODS

The fern spore sterilization method of Ford and Fay (1990) was refined for application to gemmae and a 0.1M solution of sodium hypochlorite low in



a b c

FIG. 1. Construction and use of filter paper packets. a) Folds necessary to generate packet; numbers indicate fold sequence; gemmae placed on hatched region only. b) Stapled packet; after sterilization the staple and top are cut off as indicated by arrows. c) Opened sterile packet, gemmae distributed throughout hatched region.

bromine (Merck Ltd., Product no. 23039) used (Adams, pers. comm.) as follows: 7cm Whatman 541 hardened ashless filter papers were folded to form filter paper packets (FPPs, see Fig. 1). Petri dishes (9 cm) were lined with filter paper and moistened with distilled water. Gemmiferous clumps of *Trichomanes speciosum* gametophyte were extracted from (soil-based) source cultures (Rumsey, 1994), inverted and dabbed over the whole of the filter paper surface. This released and dispersed gemmae. A pre-folded FPP was opened and placed in a petri dish; the central area (see hatched area on Fig. 1a) was dampened with sterilized distilled water, but not soaked. Using a dissecting microscope and fine forceps, gemmae were carefully removed from the source petri dish and placed in the central region of the open FPP. Damaged or germinated gemmae were not used; those selected consisted of 4–12 cells. Each FPP was refolded and stapled at the end furthest from the gemmae, and placed in a dish containing sterilized distilled water to avoid desiccation while others were prepared. Just before surface sterilization, the FPPs were gently squeezed to expel trapped air. Up to 18 FPPs per run were submerged in 300 ml surface sterilant in a 500ml wide-necked conical flask. FPPs were agitated in the solution via a large magnetic stirrer bar and placing on a stirrer.

The remainder of the procedure was performed in a laminar flow unit. Scissors and forceps were flame sterilized before use.

After 2, 5, or 10 min, the sterilant packets were removed one at a time using large forceps. Excess sterilant was carefully squeezed out and the packet dropped into a 500ml conical flask containing sterilized distilled water. All packets were then washed for 10 min, agitating as before. This post-sterilization procedure of removing, squeezing, and washing packets was repeated three times.

After the final wash, single packets were removed and as much water as possible expelled from the FPP. The stapled end was cut off with scissors and discarded (Fig. 1b). FPPs were opened using two pairs of small forceps. Gemmae were spread all over the exposed surface of the FPPs when opened (Fig.

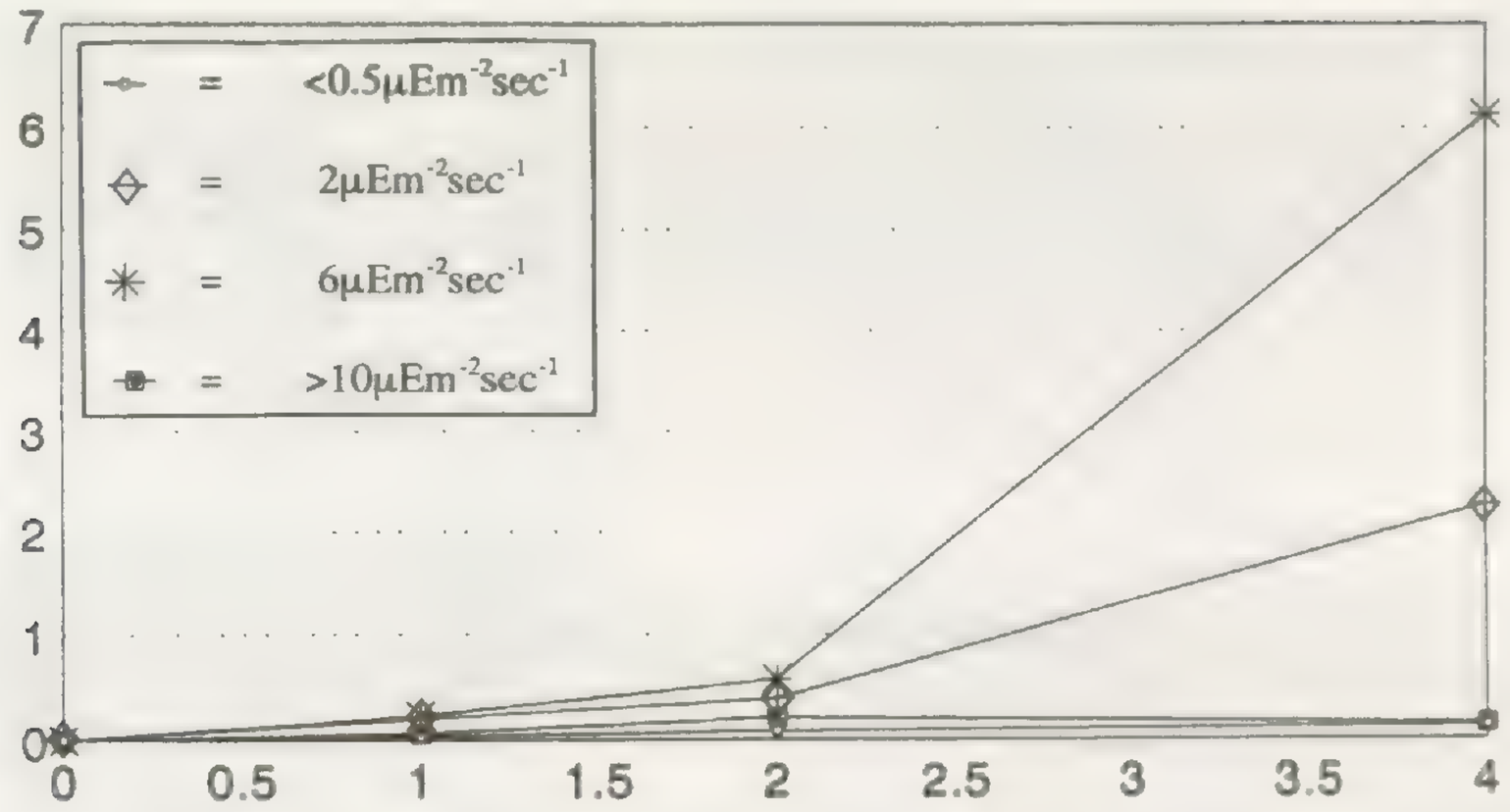


FIG. 2. Graph showing change in cell number per original cell of *Trichomanes speciosum* gemmae cultured at 12°C in 4 different PAR levels.

1c), so care was taken not to let water drop off the opened FPP, as this contained most of the gemmae. A petri dish of medium was opened, and the whole open FPP placed face down on the agar surface. The FPP was patted gently so that it was flat and in complete contact with the surface. The FPP was peeled back and forth several times to release gemmae onto the surface, and the FPP discarded. The petri dish lid was replaced, sealed with plastic film (Nescofilm), labeled, and incubated at 12° or 20°C, with constant illumination provided by fluorescent daylight tubes. Four different light intensities were generated by covering the dishes with 0, 4, 8, and 32 sheets of fine mesh muslin. Photosynthetically available radiation (PAR) values at the level of the dishes were measured.

Defined media tested included Moore's (1903) and Hoagland's (Reuter, 1953) made up at full and one tenth strength, with and without 2% sucrose (to help reveal contaminants), solidified with 0.8% Difco Bacto agar. Cultures were observed with a dissecting microscope after 1, 2, and 4 months, and the numbers of cells per original cell of all the gemmae counted (8–16 gemmae per culture).

RESULTS

Gemmae were unharmed by surface sterilization for 2 min; they germinated and grew well, but most cultures became contaminated with microorganisms. The attachment cell of each gemma died as a result of the 5 min surface sterilization procedure, but the remaining cells were unharmed and fungal contamination occurred very infrequently. A 10 min surface sterilization step reduced contamination to negligible levels, but caused the death of both terminal cells in addition to the attachment cell. A 5 min period was therefore routinely adopted for *Trichomanes speciosum* gemmae.

Gemmae were bleached and their cells became swollen at $>10\mu Em^{-2}sec^{-1}$; very little growth was observed at this level of PAR or below $0.5\mu Em^{-2}sec^{-1}$ (see Figs. 2, 3). Cultures maintained at 2 or $6\mu Em^{-2}sec^{-1}$ germinated and grew,

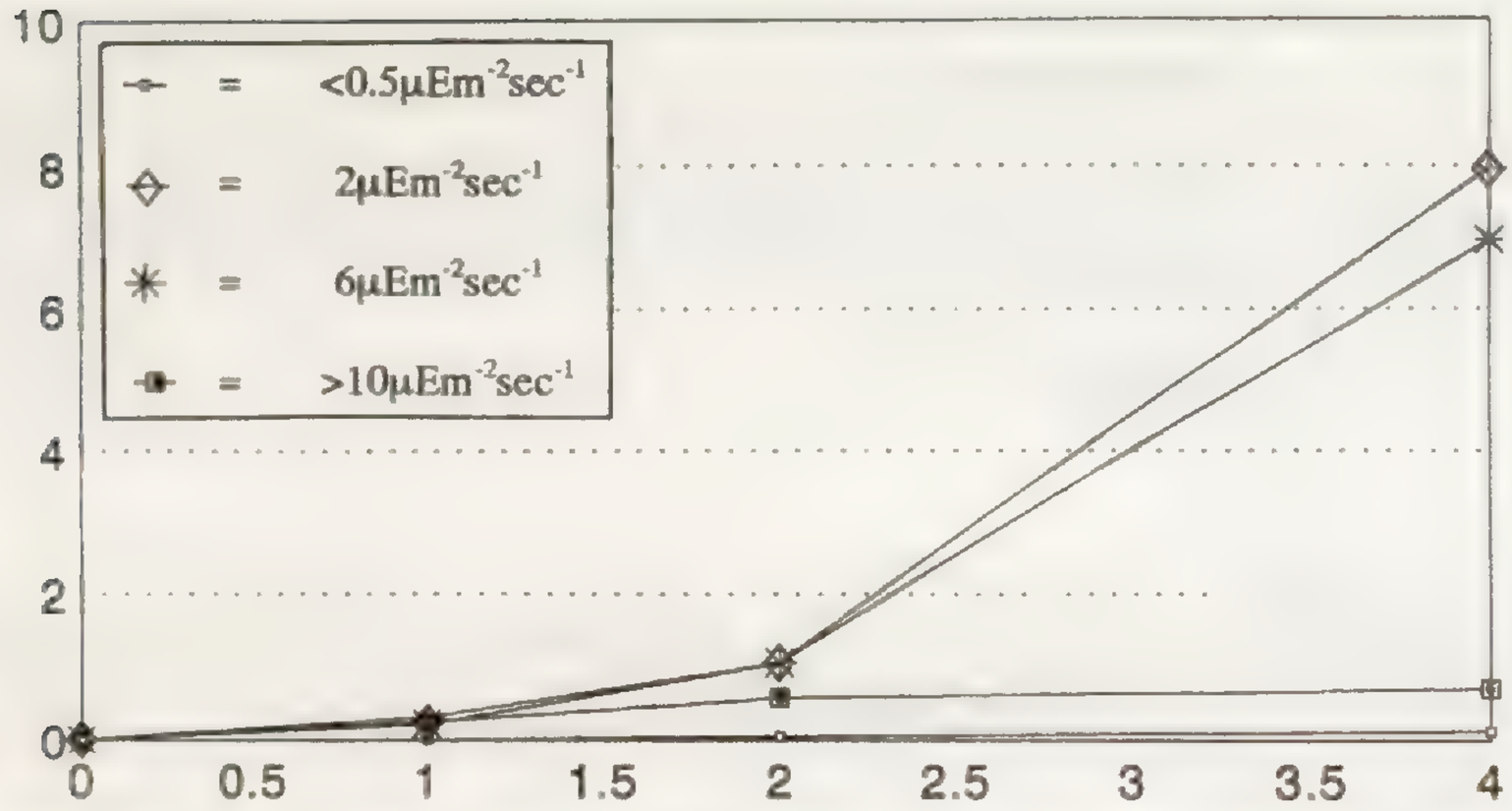


FIG. 3. Graph showing change in cell number per original cell of *Trichomanes speciosum* gemmae cultured at 20°C in 4 different PAR levels.

but those kept at 20°C produced greater biomass than those maintained at 12°C (see Figs. 2, 3).

Gemmae germinated and grew on all the media tested, but one tenth strength Hoagland's with sucrose supported the most rapid growth. Gametophytes produced from gemmae generated gemmae themselves after 8 months of culture. Undisturbed cultures remained healthy and contaminant free for 50 months or more, during which time they established the three dimensional mat-like form of gametophytes seen in the wild (see Figs. 4–6).

DISCUSSION

The availability of a surface-sterilizing procedure for *Trichomanes speciosum* gemmae obviates the need for collection of spores to initiate gametophyte cultures. The method is simple, effective, and applicable to a wide range of gemmiferous plants. Although the large multicellular gemmae of the bryophyte *Tetraphis* do not survive the procedures described herein, the smaller single-celled gemmae of *Calypogeia* and those of the ferns *Trichomanes bauerianum* Endl., *T. cuspidatum* Willd., and *Vittaria* spp. produce contaminant-free cultures that grow and generate their own gemmae faster than *Trichomanes speciosum* (Raine, 1994).

Moist, sterilized gemmae were not stored in the refrigerator during this study, but Dassler (pers. comm.) has observed healthy detached gemmae of *Callistopteris* on cultivated bryophyte mats collected two years earlier, which suggests that they may remain viable for considerably longer than dried spores of the Hymenophyllaceae. Wet storage of gemmae may be an effective way to increase the longevity of stored material of filmy ferns, providing a parallel with the improved longevity found for wet-stored spores of both green- and nongreen-spored ferns (Lindsay et al., 1992).

Once established, aseptic cultures generated from gemmae produce a potentially continuous and immortal supply of material, as they can simply be inverted over fresh dishes of media in a laminar flow unit and tapped to release

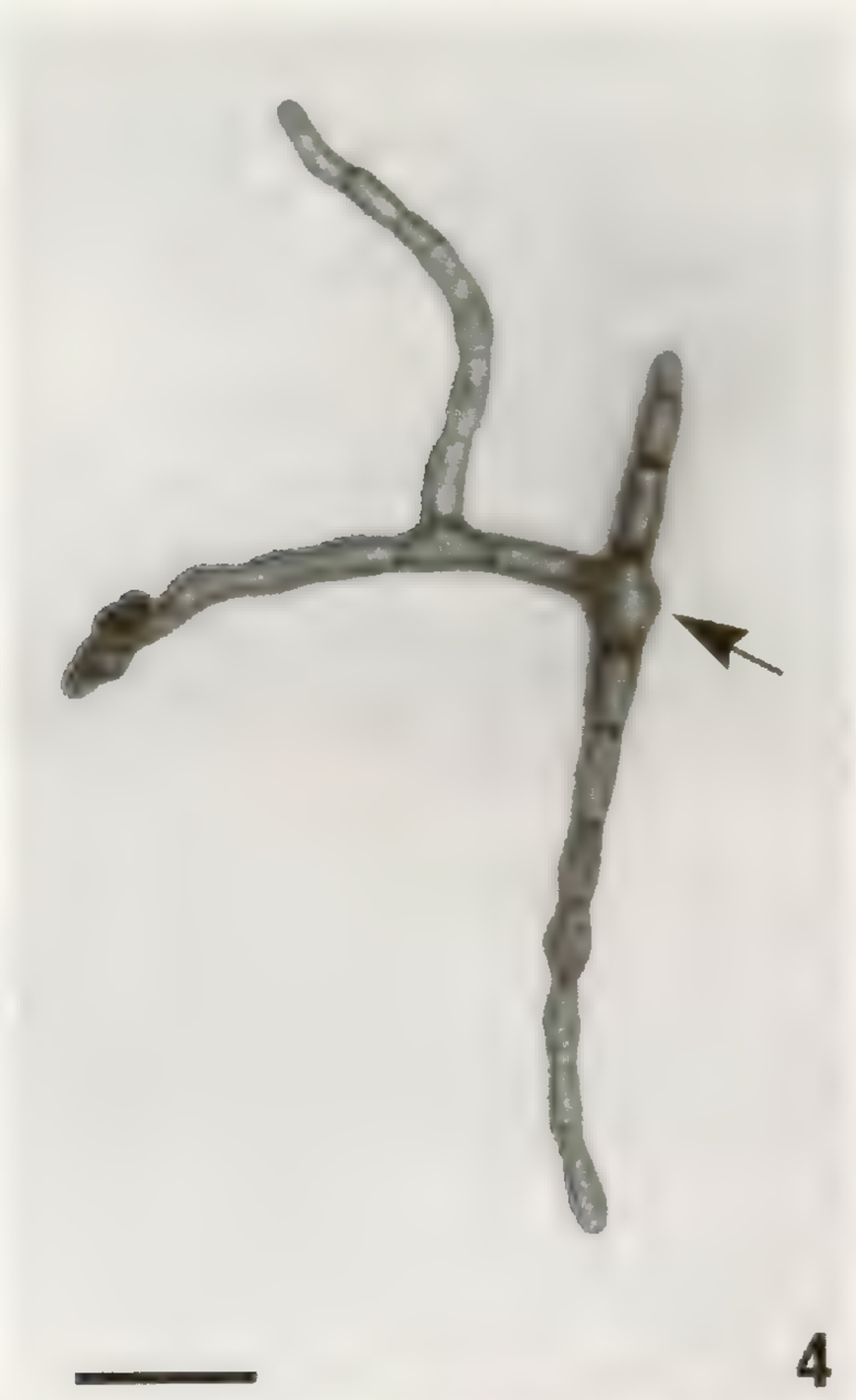


FIG. 4. Germinated gemma of *Trichomanes speciosum* in aseptic culture. Arrow indicates dehiscence scar of original gemma. Bar=100 μm.

FIG. 5. As in Fig. 4, but several weeks older; rhizoids and the characteristic three dimensional mat have started to form. Bar=100 μm.

FIG. 6. More than two year old aseptic culture of *Trichomanes speciosum* initiated from gemmae showing mature form of mat. Arrow indicates gemma production. Bar=300 μm.

gemmae onto the new surfaces. This method therefore provides the first opportunity for experiments involving fully defined media and conditions using this endangered species. It is now, for example, possible to test a vast range of environmental regimes and media supplements for their influence upon growth and gametangial induction.

Growth of cultures was extremely poor when dishes were maintained at temperatures and PAR levels similar to those recorded for *Trichomanes speciosum* in British field sites (Rumsey, 1994). This implies that growth of this species is severely limited by current climatic conditions in Britain, that recruitment of new sites is probably infrequent, and that sites with large areas of gametophyte cover represent many decades of growth.

ACKNOWLEDGMENTS

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Aspects of Spore Dispersal in *Selaginella*

Dedicated to the memory of Prof. Dr. Tadeus Reichstein

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ABSTRACT.—The evolution of heterospory changed the conditions for spore dispersal. Assuming wind as the dispersal agent, microspores will be dispersed to greater distances than megaspores. We investigated aspects of spore size and sculpture as well as spore dispersal under calm conditions and under the effect of artificial wind of some *Selaginella* species. We found that differences between the species existed that were correlated with active or passive release of micro- and megaspores. The dispersal efficacy changed drastically under the effect of wind, showing differences between the species. There was no support for the hypothesis of synaptospory between micro- and megaspores during active dispersal. Evidence suggests that active spore dispersal and wind may enhance outbreeding. More detailed investigations may reveal species specific correlations between life history traits, breeding systems, and spore dispersal.

Heterospory and dioecy of gametophytes evolved independently in different clades of the pteridophytes. The separation of female megaspores and male microspores changed the conditions of spore dispersal. In homosporous, wind dispersed ferns the probability of being transported a certain distance is much the same for each spore. Assuming that in heterosporous pteridophytes wind dispersal is still dominant, the smaller and lighter microspores are likely to be dispersed to longer distances than the larger and heavier megaspores (Tryon and Lugardon, 1991). The dispersal distances of male and female gametophytes thus differ. Heterospory also affects the production of spores. Because of higher energetic investments, the number of megaspores per individual plant is reduced in comparison to the number of microspores (Tryon and Tryon, 1982). As a consequence, the dispersal ecology of heterosporous pteridophytes is likely to differ from that of homosporous ferns (Cousens, 1988).

The heterosporous genus *Selaginella* P. Beauv. is widespread and contains numerous species (Jermy, 1990). The efficacy of their active spore dispersal mechanisms was first investigated by Goebel (1901). He showed that the megasporangium ejects the megaspores in a manner quite different from the microspore release mechanism of the microsporangium (Goebel, 1901). He gave a detailed description of the mechanism of megaspore release that was recently rediscovered by Page (1989). The active megaspore discharge mechanism was termed "compression and slingshot ejection" (Page, 1989). Studies by Straka

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(1962), Somers (1982), and Koller and Scheckler (1986) revealed that variations in release mechanisms of microspores exist. Passive spore release as well as active spore release were found. Both types can occur under conditions of calm or wind. Active spore release is due to sudden movements of the microsporangium walls caused by changes of the cell turgor during desiccation.

A possible function of spore sculpture in respect to dispersal, namely synaptospory, was emphasized by Kramer (1977). Primary synaptospory means that diaspores are dispersed together straight away from the source plant, whereas secondary synaptospory describes the sticking together of diaspores after the dispersal of single spores. Spore sculptures may enhance synaptospory. A special type of synaptospory may be found in heterosporous ferns, i.e., the dispersal of units consisting of micro- and megaspores.

The ecological consequences of the different above-mentioned spore release and dispersal mechanisms have not yet been studied. In the present investigation we compare the spore dispersal of five species of *Selaginella*. We address the following questions: 1) Are there morphological characteristics of the micro- and megasporangia and/or the micro- and megaspores that support dispersal mechanisms? 2) Do the species differ with respect to release modes? 3) Do different release mechanisms of micro- and megaspores result in differences in dispersal distances? 4) How do these distances change under the effect of wind? 5) Does primary synaptospory between micro- and megaspores occur?

Methods and Materials

The Species.—The following species cultivated in the Botanical Garden of the University of Zürich were investigated: *Selaginella anceps* (C. Presl) C. Presl, *S. kraussiana* A. Braun, *S. lepidophylla* Spring, *S. martensii* Spring, and *S. pallescens* (C. Presl) Spring. The species were identified using Vareschi (1968) and Alston et al. (1981).

Morphological Traits of Sporangia and Spores.—The size (largest diameter) and the surface sculpture of the outer spore wall of micro- and megaspores were determined using a light microscope and SEM. Sizes of both spore types were measured roughly; a statistically relevant measurement of spore sizes was not intended. The arrangement of micro- and megasporangia on morphological units of plants (see below) was determined using a dissecting microscope. Sporangial arrangement of each species was assigned to the four types given by Horner and Arnott (1963). Type I: basal megasporangiate zone and superior microsporangiate zone; Type II: two rows of megasporangia and two rows of microsporangia within each strobilus; Type II': strobili with two rows of microsporangia and two rows containing both mega- and microsporangia; Type III: wholly megasporangiate strobili.

Spore Release.—The opening of sporangia and the manner of active and passive spore release were observed visually in all five species under a dissecting

microscope. Micro- and megaspore release in *S. lepidophylla* and *S. martensii* were studied additionally with a video camera fitted with a macro lens.

SPORE DISPERSAL.—Because of the branching morphology of *Selaginella,* we used morphological units of plants as spore sources. These were vertically oriented, frond-like parts of fertile shoots about 40 cm long in *S. anceps, S. martensii,* and *S. pallescens.* The units were short, horizontally oriented shoots of *S. lepidophylla* and short, horizontal parts of shoots with some erect strobili in *S. kraussiana.* As a consequence, the spores were not released at even heights in all experiments. This experimental design was chosen in order to get an impression of the pattern of spore dispersal distances under natural conditions.

We arranged two types of experiments, one in calm and one with moderate wind. Both experiments were carried out in a calm room. Room temperature was constant at 20°C, which allowed slow desiccation of the morphological units, a basic requirement for spore dispersal. The floor of the room was covered with black paper.

Morphological units bearing ripe micro- and megasporangia of *S. anceps, S. martensii,* and *S. pallescens* were placed erectly with the strobili at an approximate height of 30 cm above surface. Eight rows, each of 59 cm length, of microscope slides covered with double-sided clear sticky tape were laid at angles of 45° around the spore source. The experiments were conducted for twelve hours. Then, the diaspores (single microspores, tetrads of microspores or single megaspores) were counted in each centimeter of each row on an area of 16 mm^2 under a light microscope. The numbers of diaspores were extrapolated to 1 cm^2, and the mean per cm distance from the spore source of the eight rows was calculated. For *S. kraussiana* and *S. lepidophylla,* a similar experiment was performed, but because of the much smaller morphological units of these plants (strobili at a height of 3–4 cm above the surface), they were carried out in large plastic boxes, and the 8 rows around the spore source had a length of 14 cm.

We also investigated the spore dispersal of *S. anceps, S. kraussiana,* and *S. martensii* under the influence of wind. Again, morphological units of these species were placed in a calm room. Five rows of microscope slides with double-sided clear sticky tape, each 119 cm long, were laid at angles of 45° in a semicircle on one side of the spore source. On the other side, a small electric propeller (König AG, Zürich, type 316; diameter of the propeller = 19 cm) was placed at a distance of 1 m. This propeller did not produce a linear wind flow, but an air current with turbulence, which we believe to be more representative for natural wind conditions. The wind speed was measured at the position of the strobili using a digital data logger (Squirrel, Grant Ltd., Britain). In all experiments a moderate mean wind speed of 1.16 m/s was adjusted. The experiments again were conducted for twelve hours. Data analysis was carried out in the manner described above.

SYNAPTOSPORY.—All experiments were carefully checked for eventual synaptospory between micro- and megaspores.

TABLE 1. Morphological characteristics of microspores, megaspores, and strobili of *Selaginella anceps, S. kraussiana, S. lepidophylla, S. martensii,* and *S. pallescens* used in dispersal experiments. Arrangement of sporangia within strobili according to Horner and Arnott (1963; for explanations see text).

Species	*Selaginella anceps*	*Selaginella kraussiana*	*Selaginella lepidophylla*	*Selaginella martensii*	*Selaginella pallescens*
Microspore dispersal units, and their size	~ 40 μm tetrads	~ 40 μm singles	~ 50 μm tetrads	~ 40 μm tetrads	~ 30 μm singles or tetrads
Surface of microspores	obtuse cones	spiny	burlike structured	finely prickly	papillate
Size of megaspores	~ 300 μm (with flange)	~ 620 μm	~ 300 μm	~ 380 μm	~ 330 μm
Surface of megaspores	with large equatorial flange	distantly reticulate	feltlike	feltlike	narrowly reticulate
Release of microspores	active	passive	active	active	active
Release of megaspores	passive	active	active	active	active
Arrangement of sporangia within strobili	type I	type I	type II	type I	type II′
Approximative height of sporangia above surface	~ 30 cm within the plane of the shoot	~ 3 cm vertical	~ 3 cm horizontal	~ 25 cm within the plane of the shoot	~ 25 cm within the plane of the shoot

RESULTS

CHARACTERIZATION OF MICRO- AND MEGASPORES.—The investigated species of *Selaginella* had megaspores that were 6–16 times larger in diameter than the dispersal units of the microspores, i.e., single microspores or microspore tetrads (Table 1). The largest megaspores were found in *S. kraussiana* (ca. 620 μm in diameter). In *S. anceps, S. lepidophylla,* and *S. martensii* microspores were dispersed as tetrads (Table 1). *Selaginella kraussiana* dispersed single microspores, whereas in *S. pallescens* both single microspores and tetrads occurred. It is noteworthy that the single microspores of *S. kraussiana* approximately reached the size of the microspore tetrads of the other species.

The surfaces of both types of spores showed distinct characterizations (Table 1, Fig. 1). In *S. anceps,* the megaspores were characterized by a large equatorial flange. The microspores of *S. kraussiana* were extremely spiny. In *S. lepidophylla,* the surfaces of the microspore tetrads had burlike structures at the edges that seemed to be complementary to the feltlike surfaces of the megaspores. A similar case was found in *S. martensii,* but here the microspore tetrads were finely prickly. In two of the investigated species, surface structures of the spores were documented, which principally allowed synaptospory between micro- and megaspores, whereas the surface structures of all five species allowed synaptospory of microspores. In the dispersal experiments (see

below), we sometimes observed groups of microspore tetrads dispersed as one unit.

ARRANGEMENT OF MICRO- AND MEGASPORES WITHIN THE STROBILI.—We found three of the four types of sporangial arrangement described by Horner and Arnott (1963). Type I, with megasporangia at the base of the strobilus, occured in *S. kraussiana, S. martensii,* and *S. anceps.* Type II, with entirely vertical rows of either micro- or megasporangia, was documented in *S. lepidophylla,* and type II', with vertical, but intermingled rows of both sporangial types, was found in *S. pallescens.*

The strobili usually were oriented within the plane of the morphological unit, i.e., the shoot (Table 1). Depending on the architecture of the species, the sporangia were oriented strictly vertically or horizontally, the latter observed in *S. kraussiana,* with tower-like strobili.

SPORE RELEASE.—The height above surface at which the spores were released changed among the species and clearly affected the dispersal distances. The strobili of *S. kraussiana* and *S. lepidophylla* were at heights of only 3 cm above surface (Table 1).

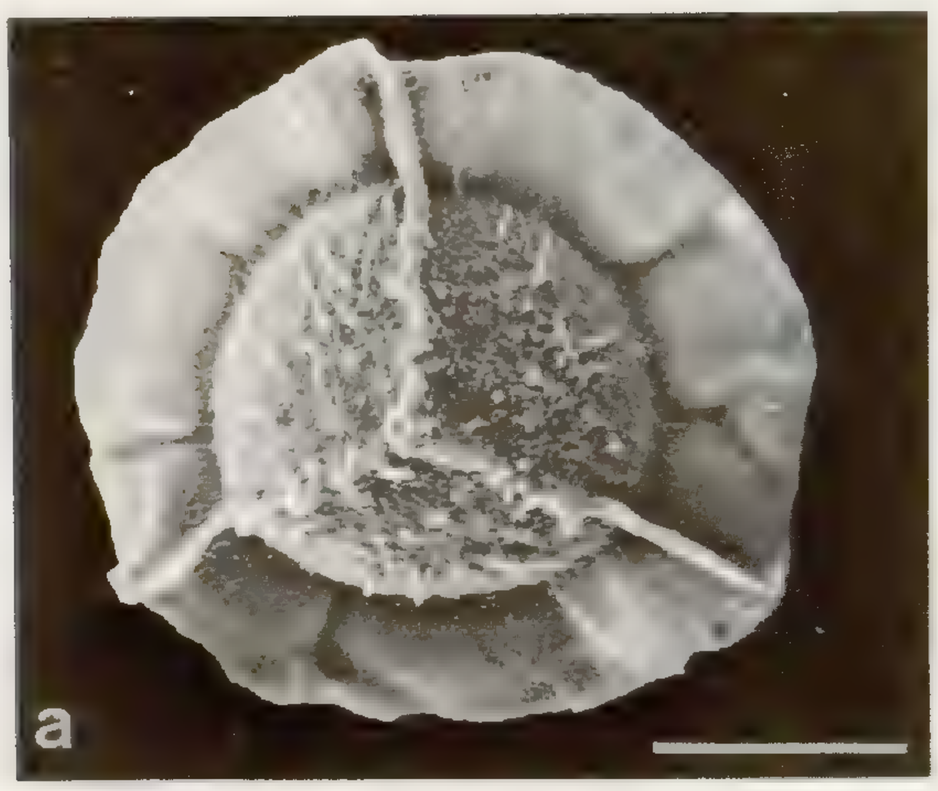
Microspores were either actively or passively dispersed (Table 1). In *S. kraussiana,* the microsporangium opened along a preformed dehiscence line, and the microspores were passively dispersed (as described in Koller and Scheckler, 1986). The two valves of the sporangial wall opened during desiccation. Subsequently, they remained open. The microspores were exposed to the air and, as a consequence, the tapetal secretion on the inner surface of the valves dried. Some spores fell out and some were dispersed by wind.

The microsporangia of the other four species dispersed their spores actively (Table 1). The sporangium started to open at the distal end and continued opening along a preformed dehiscent line. The two valves of the microsporangia spread and their borders bent backwards. When the angle between the valves reached about 150°, the sporangium suddenly closed, actively dispersing the microspores.

In *S. anceps,* the megaspores fell passively out of the open sporangium and were not dispersed by a slingshot mechanism (Table 1). Therefore, in *S. anceps,* only the microspores were actively dispersed.

In the other four investigated species of *Selaginella,* the megasporangium opened in the manner described by Page (1989). During desiccation, the valves spread until an angle of about 150° was reached. The megaspores were then separate from each other. The two outer megaspores, i.e., the ones lying at the borders of the valves, were both located in hollows of the corresponding valves. The two inner megaspores were in the boat-shaped basal part of the sporangium. With a sudden movement, the valves sprang back into their original position, actively ejecting the megaspores (Table 1).

During the opening of the micro- and megasporangia the sporophylls spread to an angle of approximately 90° or even more, leaving space for spore dispersal. After spore release they closed again to an angle of 45°.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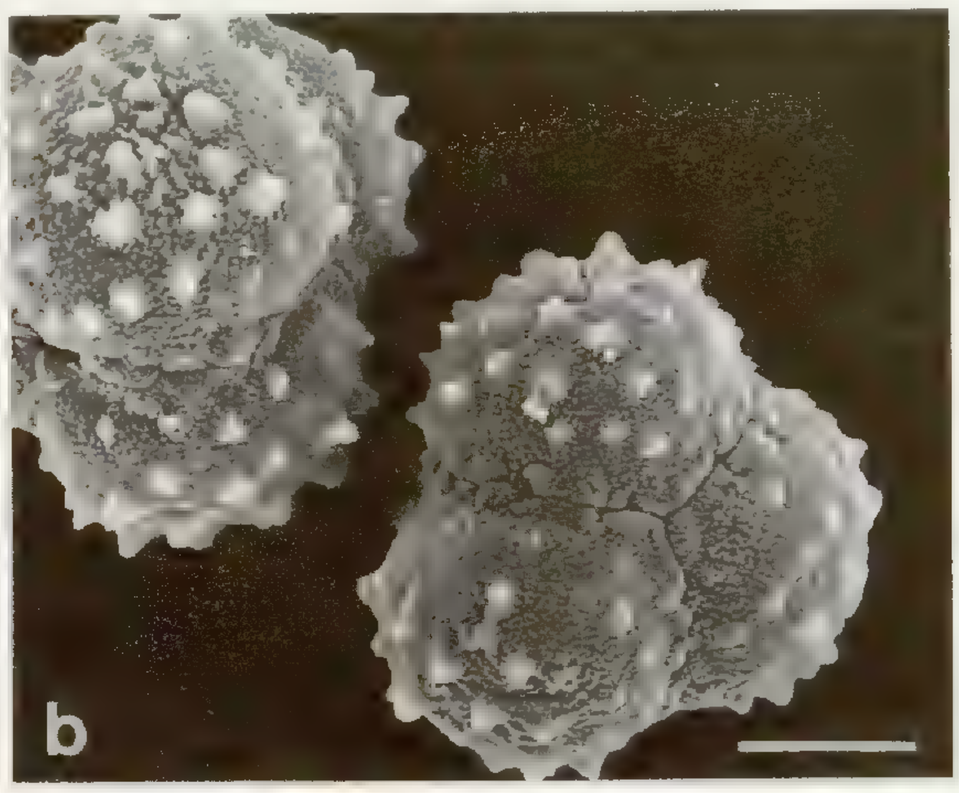
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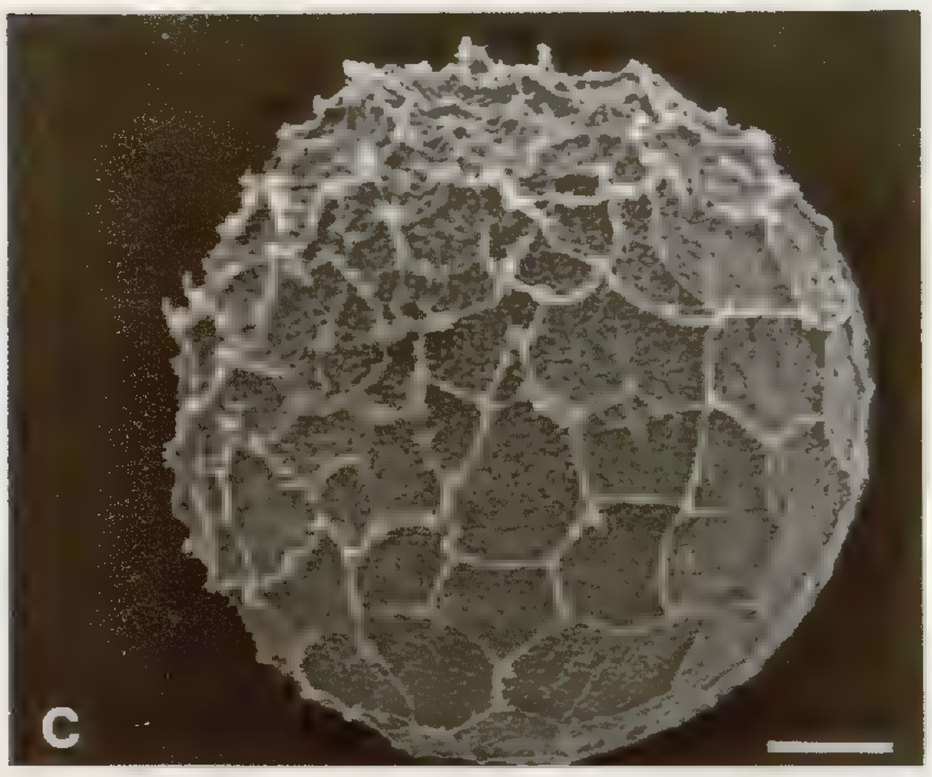
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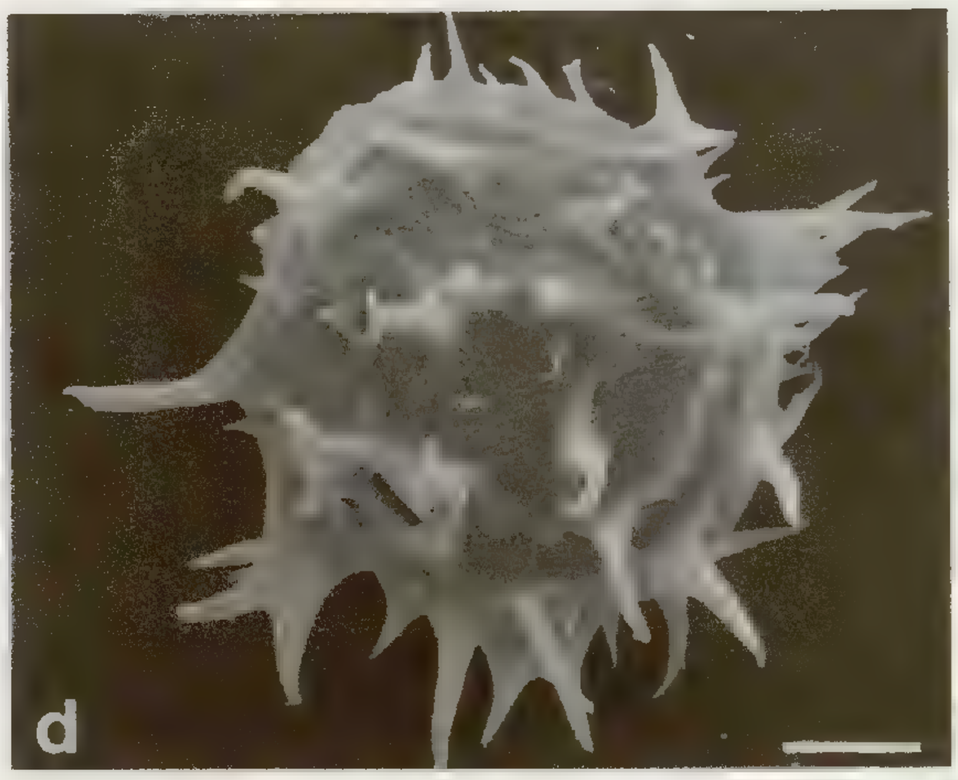
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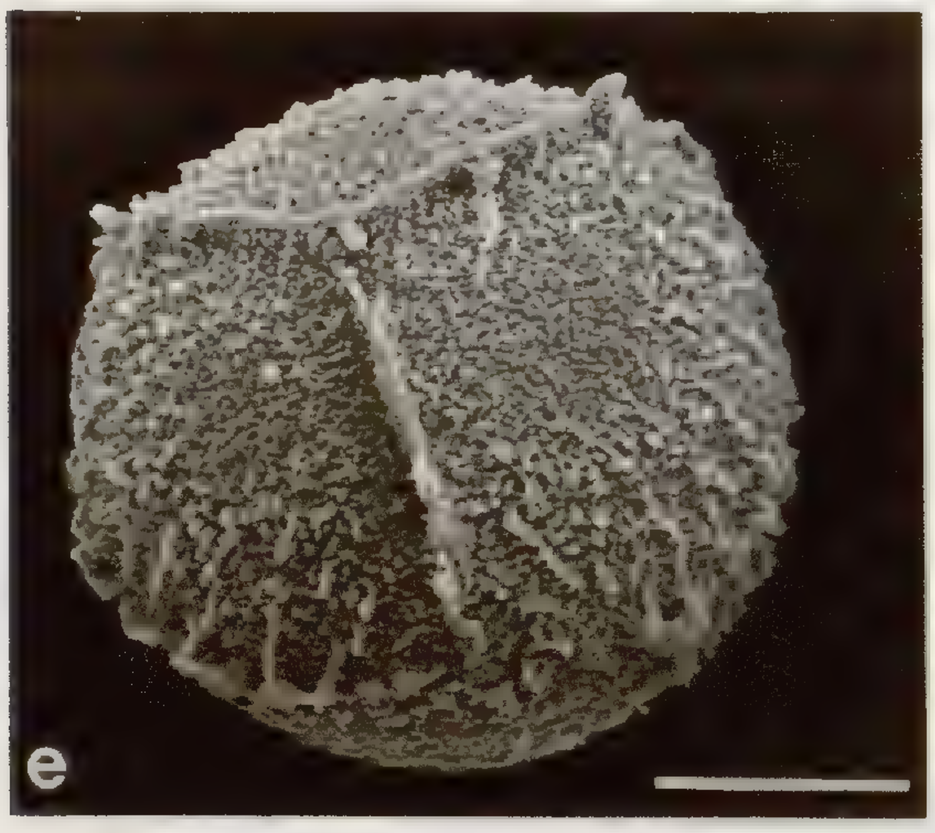
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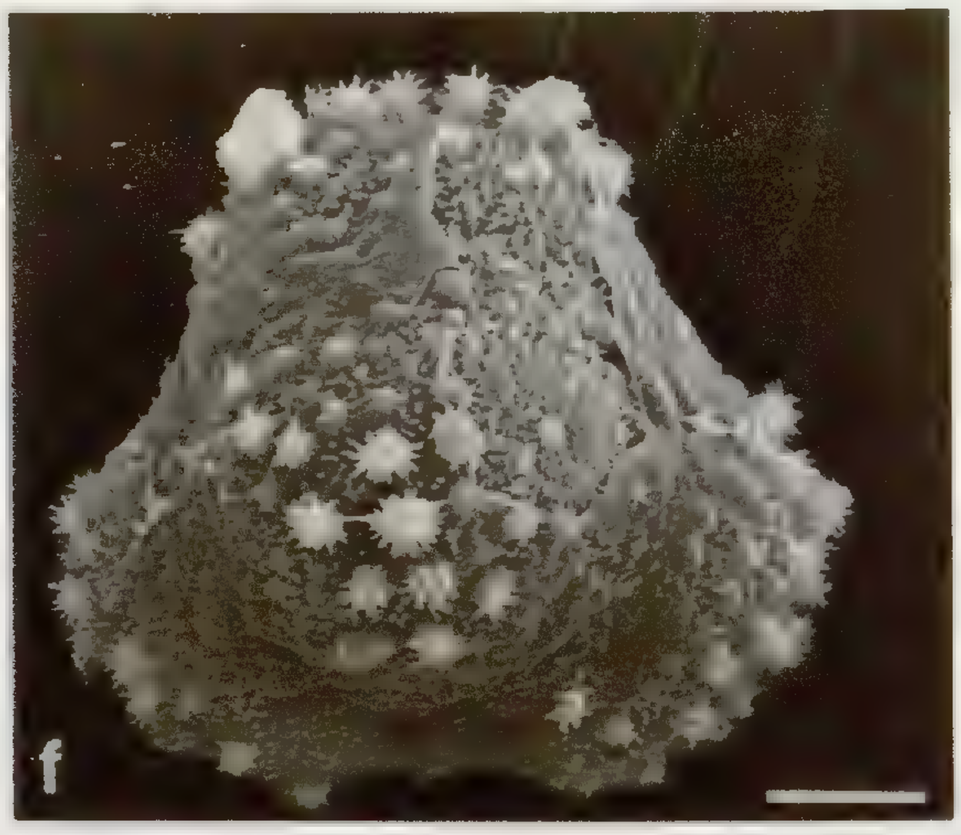
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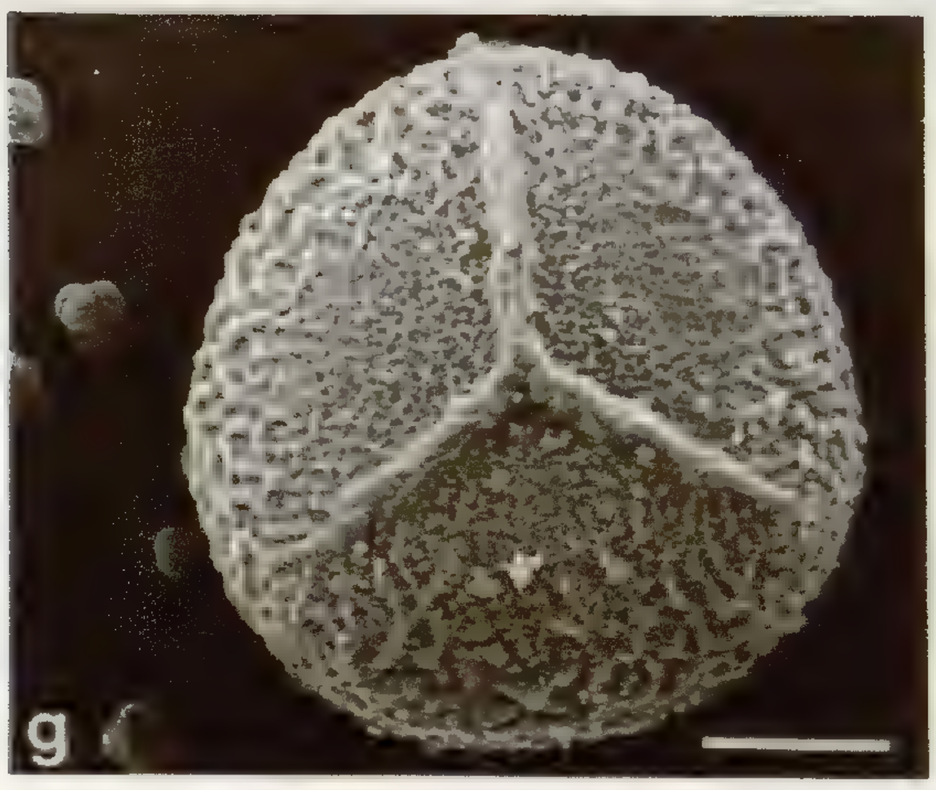
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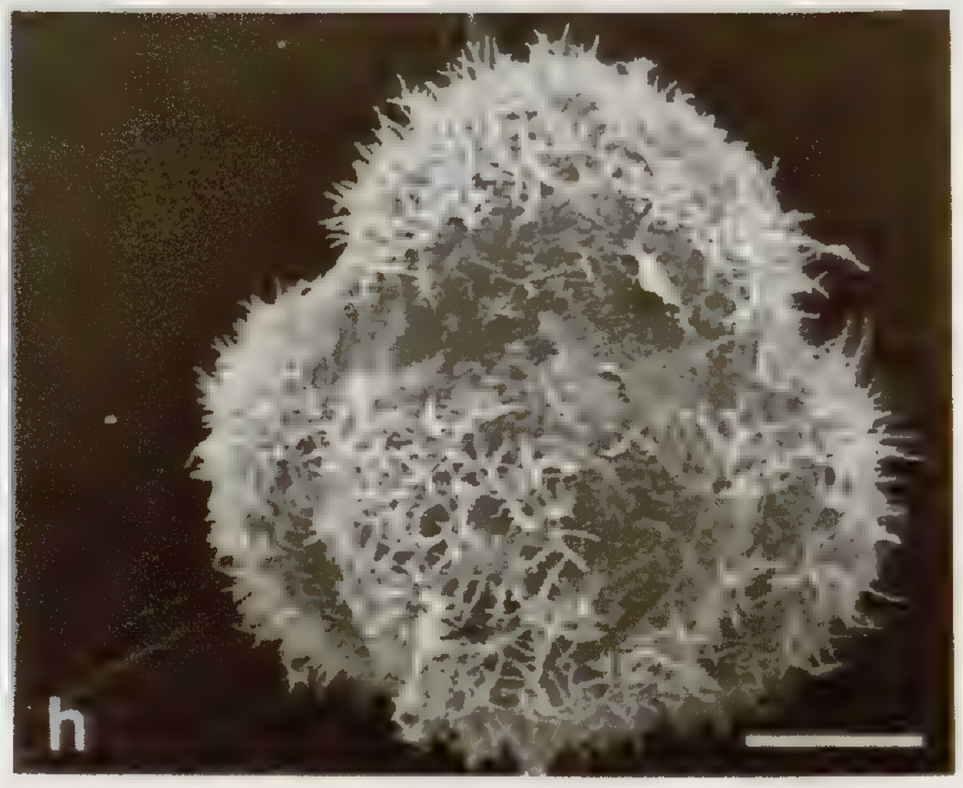
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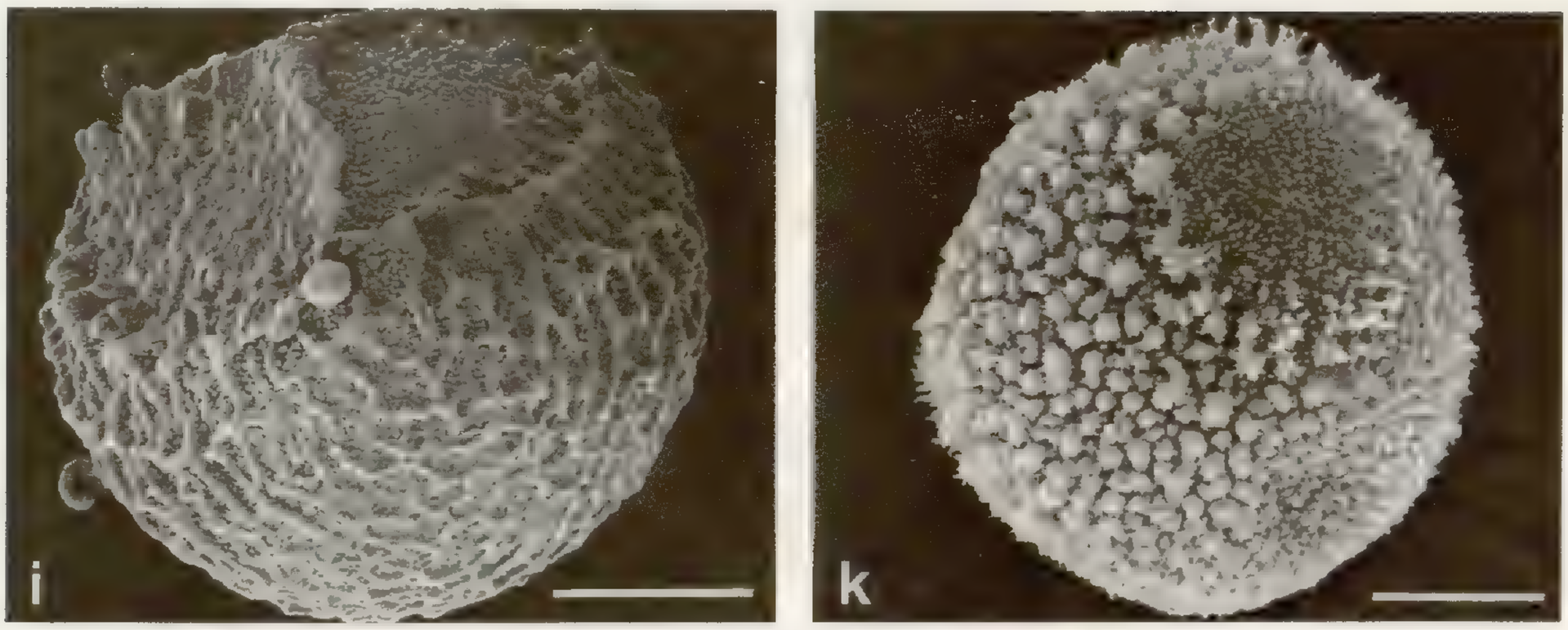

FIG. 1. Continued.

SPORE DISPERSAL.—In *S. anceps,* most megaspores were only dispersed into the close vicinity of the spore source in the experiments without wind (Fig. 2), a result that is in accordance to the passive spore release mechanism of this species. Densities of 1300 microspore tetrads (Fig. 1b) per cm^2 were found within 20 cm from the source (Fig. 2). The majority of the microspores landed here, leading to the well known leptocurtic curve of spore dispersal (Fig. 2). However, a minority was dispersed to distances up to 59 cm. Microspores were often clumped together, forming groups of sometimes up to 100 tetrads. This seemed to be due mainly to a tapetal secretion on the surfaces of the spores. Under the influence of a moderate wind of 1.16 m/s, an almost uniform dispersal curve was observed, exhibiting no obvious peaks (Fig. 2). The abundance of microspores within the range of the experiment decreased only slightly with increasing distance from the source. The megaspores also reached greater distances—up to 69 cm in the experiment with wind—possibly assisted by their large equatorial flange (Fig. 1a).

Similar results were obtained for the microspore tetrads in the experiments with *S. martensii* (Fig. 2). Near the spore source, we counted about 920 microspore tetrads per cm^2. In marked contrast to *S. anceps,* the actively released megaspores of *S. martensii* reached similar distances in calm conditions and under the effect of wind (Fig. 2), i.e., up to 59 cm from the spore source. Under calm conditions in the experiment with *S. pallescens* (Fig. 2), the results of micro- and megaspores corresponded to the dispersal curves found in *S. martensii* (Fig. 2).

Selaginella lepidophylla and *S. kraussiana* differed from the above de-

←

FIG. 1. SEM micrographs of microspores and megaspores of *Selaginella* species. a) proximal view of a megaspore of *S. anceps* with equatorial flange; b) microspore tetrads of *S. anceps*; c) equatorial view of a megaspore of *S. kraussiana*; d) single, spiny microspore of *S. kraussiana*; e) proximal view of a megaspore of *S. lepidophylla*; f) microspore tetrad of *S. lepidophylla*; g) proximal view of a megaspore of *S. martensii*; h) finely prickly microspore tetrad of *S. martensii*; i) equatorial view of a megaspore of *S. pallescens*; k) single microspore of *S. pallescens*. Scale bars: a, c, e, g, i = 100 μm; b, d, f, h, k = 10μm.

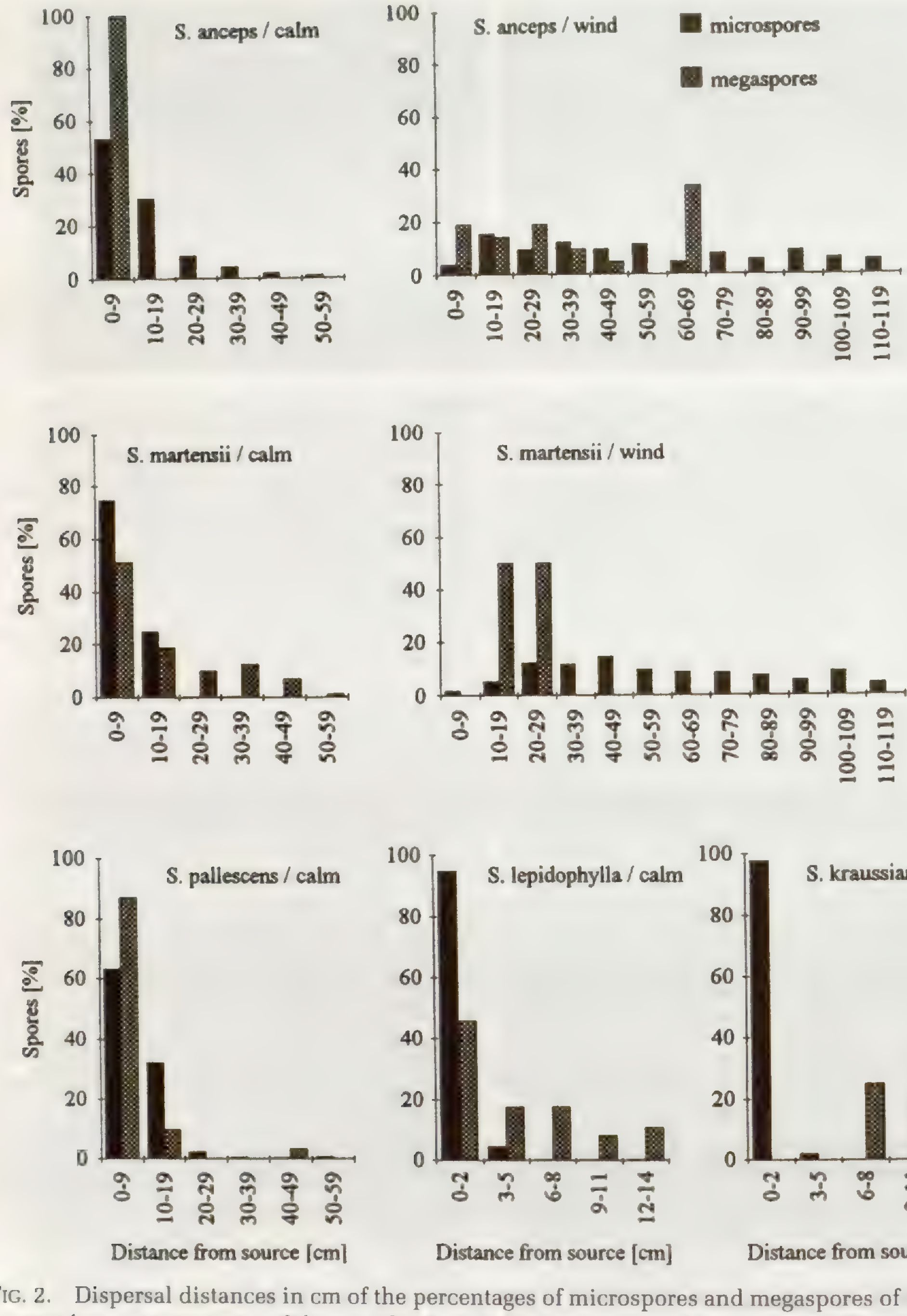

FIG. 2. Dispersal distances in cm of the percentages of microspores and megaspores of *Selaginella anceps* (mean percentages of three replicates), *S. kraussiana* (mean percentages of four replicates), *S. lepidophylla* (mean percentages of three replicates), *S. pallescens*, and *S. martensii* in calm or with wind (1.16 m/s). Note different scales of the x-axis.

scribed species in the height of strobili above ground, which was only about 3–4 cm (Table 1). The actively dispersed megaspores of both species reached distances of at least 14 cm under calm conditions, but most of the microspore tetrads in *S. lepidophylla* (Fig. 2), and most of the spiny single spores in *S.*

kraussiana were found close to the spore source (up to 2 cm; Fig. 2). In an experiment with wind, only a few micro- and megaspores were found in *S. kraussiana* and the results are therefore not shown. Because there were ripe sporangia of both types on the morphological units and because these sporangia were obviously empty after the experiment, one had to conclude that the spiny microspores were dispersed for much greater distances than the 119 cm of the experimental setup.

SYNAPTOSPORY.—In all investigations as well as in the dispersal experiments no case of primary synaptospory between micro- and megaspores was detected.

DISCUSSION

In homosporous ferns a single viable spore is theoretically sufficient to achieve a new sporophyte and to found a new population, due to intragametophytic selfing (Schneller and Holderegger, 1996). In any heterosporous species a microspore and a megaspore must be placed in close proximity to generate a new sporophyte (Kramer, 1977). The formation of heavy megaspores, compared with small, light microspores, clearly reduces the effectiveness of wind dispersal, and thus the potential for long distance dispersal. As a consequence, the dispersal units of heterosporous pteridophytes differ in primary dispersal distances: the microspores have the ability to be dispersed for long distances, whereas the megaspores are dispersed only locally.

Heterospory ultimately led to the differentiation of two types of sporangia. Earlier investigations (Goebel, 1901; Steinbrinck, 1902; Somers, 1982; Koller & Scheckler, 1986; Page, 1989) revealed the morphological, anatomical, and functional differences between micro- and megasporangia in the genus *Selaginella*. The five species of *Selaginella* investigated in the present study also exhibited marked differences in the manner of spore release. Schneller (1995) showed that differences in spore release also occur between homosporous ferns.

As hypothesized by Kramer (1977), primary synaptospory (as well as secondary) of mega- and microspores could be a mechanism to assure fertilization in heterosporous pteridophytes. If this is the case, the genetic variation within the progeny would consequently be restricted due to intergametophytic selfing. Kramer (1977) and Dahlen (1990) gave some morphological evidence in favor of this hypothesis. The surface structures of micro- and megaspores often seem to fit together in a lock and key manner. In our investigations, we also detected some morphological characteristics of the spore surfaces that suggested synaptospory. Microspores would then "ride" on ejected megaspores. However, this hypothesis of synaptospory between micro- and megaspores could not be confirmed in our investigations. Mega- and microspores were dispersed separately.

To our knowledge, no investigations exist on spore dispersal of *Selaginella* under natural conditions. Published data on dispersal distances (Mitchell,

1910; Ingold, 1939; Page, 1989) are based on laboratory observations under calm conditions. In our experiments using artificial wind, we showed that the microspores are easily transported and well spread over distances of more than 1 m from the spore source. Compared to the mean size of the spores of homosporous ferns (Kramer et al., 1995), the microspores of *Selaginella* species are smaller and thus more effectively wind dispersed. Wind also seems to have effects on megaspore dispersal (Fig. 2), especially in the case of the passively dispersed megaspores of *S. anceps,* which are characterized by a large equatorial flange. In nature, microspores would disperse far greater distances than megaspores under the influence of wind, turbulence, and squalls. Long distance dispersal of microspores thus increases the probability for outbreeding. Active mechanical dispersal and wind dispersal are not mutually exclusive. The former can additionally faciliate wind dispersal. Active spore release by the megasporangium would further enhance the possibility of cross fertilization, especially in calm, when the majority of microspores are deposited adjacent to the source (leptocurtic dispersal curve; Fig. 2) and the megaspores are dispersed greater distances. This effective active megaspore release mechanism may also increase the probability for the establishment of new sporophytes due to decreased competition among siblings.

Secondary synaptospory after spore dispersal may be relevant in promoting fertilization. The complementary spore surface sculptures of micro- and megaspores possibly play a role in the effectiveness of secondary synaptospory. A group of fertile microspores or a group of microspore tetrads in the vicinity of a megaprothallium may significantly enhance the probability of fertilization, because of its higher number of spermatozoids that are released. Water and electrostatic effects may also play a role in secondary synaptospory.

We do not know much about the breeding systems of *Selaginella* species, especially in natural populations. Simple breeding experiments showed that *S. martensii* frequently formed viable sporophytes through intergametophytic selfing (Filippini-De Giorgi, unpubl. data). In an electrophoretic study of some populations of *S. helvetica* in Switzerland, considerable genetic variation within and among populations was found (Holderegger and Schneller, unpubl. data). We believe that dispersal is an important but unfortunately neglected part of the life cycle in studies of population biology of pteridophytes. Our preliminary study on the dispersal biology of heterosporous *Selaginella* species reveals many possible correlations between morphology, surface sculpture of the outer spore wall, ecological determinants like drought or wind, breeding system, and population genetics. Further, thorough studies should include the determination of the terminal settling velocities of either spore type (see Niklas, 1992) or experimental designs that allow an investigation of lateral dispersal out from a real point-source (i.e., a single strobilus). The data gathered in our experiments had low statistical power. Therefore, large sample size experiments of spore dispersal under strictly controlled conditions, perhaps in a wind tunnel, are needed. Investigations of additional species using different methods may reveal whether inbreeding and outbreeding occur in natural populations, whether differences in the breeding system between species exist,

and whether these life history traits correlate with the dispersal mode of the species.

ACKNOWLEDGMENTS

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Levels and Patterns of Genetic Variation in *Isoëtes karstenii* with Observations on *I. palmeri*

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ABSTRACT.—Genetic variation was surveyed in eleven populations from throughout the range of the diploid *Isoëtes karstenii,* as well as in two populations of a closely related tetraploid, *I. palmeri,* using allozyme electrophoresis. Genetic variation was high in *I. karstenii* and appears to be highly structured, as evidenced by analyses of F-statistics and genetic vs. geographic distance. No fixed unique alleles were found that could distinguish between *I. karstenii* and *I. palmeri,* although one allele was observed that was unique to *I. palmeri.* These analyses confirm earlier observations suggesting low levels of interpopulational gene flow and have implications for sampling strategies.

Isoëtes karstenii A. Braun was first described based on a collection from the high-altitude páramos of Merida, Venezuela (Braun, 1862). Since that time it has variously been accepted or synonymized by subsequent researchers, yet no detailed discussions of its variation or distribution have been published. As part of continuing research aimed at characterizing the neotropical species of *Isoëtes,* we performed a survey of genetic variation in *I. karstenii* from throughout its range, as well as in a pair of populations from a single locality of a related species, *I. palmeri* H.P. Fuchs, using allozyme electrophoresis. A taxonomic revision of the species complex to which these taxa belong is currently in preparation (Small and Hickey, in prep.).

The utility of allozyme data in plant systematics in general is well documented (Gottlieb, 1977, 1982; Crawford, 1985; Weeden and Wendel, 1989), and has been applied profitably to studies of *Isoëtes* (Taylor et al., 1985; Hickey et al., 1989a,b; Duff and Evans, 1992). In *Isoëtes,* allozymes have been useful in delimiting species, assessing parentage of polyploid taxa and hybrids, and hypothesizing phylogenetic relationships within species complexes.

MATERIALS AND METHODS

Materials and methods for enzyme electrophoresis follow standard protocols derived from published electrophoretic and staining procedures (Soltis et al., 1983; Werth, 1985). Individual specimens from eleven populations from throughout the range of the diploid *I. karstenii* and two populations of the tetraploid *I. palmeri* (Small, 1994) were included in the analyses (see Table 1 for locality, number of individuals sampled, and voucher information). Individuals subjected to electrophoresis were grown in a cold room at ca. 4°C under a 16:8 hr light:dark cycle, fully submerged, and potted in fine quartz sand or in a mixture of 2 parts sphagnum peat: 3 parts quartz sand, as sug-

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gested by Taylor and Luebke (1986). Samples for electrophoresis consisted of leaf tips ca. 1–2 cm long that were collected either the night before or immediately prior to electrophoresis; no differences were detected between samples collected the night before and those collected immediately prior to electrophoresis. Leaf samples were ground over ice in "microbuffer" (Werth, 1985, Appendix 1), and the resulting homogenate absorbed into wicks of Whatman 3 MM filter paper that were inserted into starch gels (15% w:v). Ten enzyme systems were resolved using five different buffer systems (several enzymes were stained on multiple buffer systems): glutamate dehydrogenase (GDH), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH) were resolved on a Tris-citrate pH 8.0 buffer (Werth, 1985); isocitrate dehydrogenase (IDH), 6PGDH, phosphoglucomutase (PGM), SKDH, and triosephosphate isomerase (TPI) were resolved on buffer system 5 (Soltis et al., 1983); IDH was resolved on buffer system 11 (Soltis et al., 1983); leucine aminopeptidase (LAP), TPI, and non-specific protein (NSP; i.e., RUBISCO, Wendel and Weeden, 1989) were resolved on a LiOH buffer system (Werth, 1985). Allozyme phenotypes were documented by photographing the gel slices when fully developed. Assignment of allelic designations to individuals run on different gels was performed both by including a sample from a control plant on each gel (*I. flaccida* A. Braun), and by running samples from different gels with the same putative alleles on a "lineup" gel.

Allozyme phenotypes were scored for each individual at twelve loci and interpreted genetically according to standard allozyme analysis based on known quaternary structure and cellular compartmentalization (Weeden and Wendel, 1989) and past experience with interpretation of *Isoëtes* allozymes (Hickey et al., 1989a,b; Taylor and Hickey, 1992). The most anodally migrating isozyme was arbitrarily designated as locus one, the next most anodally migrating, as locus two, and likewise, the most anodally migrating allozyme at each locus was arbitrarily assigned the designation *a*, the next most anodally migrating allozyme *b*, etc. Genotype frequencies (allele frequencies for *I. palmeri*) for each population were calculated and analyzed using the BIOSYS-1 software package (Swofford and Selander, 1981). Measures of genetic distance of Nei (1972, 1978) and Rogers (1972), and genetic similarities of Nei (1972, 1978) and Rogers (1972) were computed from the allele frequency data and distance matrices constructed. Measures of genetic variability (number of alleles per locus, percentage of polymorphic loci) and F-statistics were calculated. Phenograms of population relationships were constructed by the unweighted pair group method with arithmetic averaging (UPGMA; Sneath and Sokal, 1973) using the genetic distance and similarity measures previously calculated. Additionally, for *I. karstenii*, geographic distance vs. genetic distance was plotted for all pairwise comparisons of populations and a correlation coefficient calculated.

RESULTS

In *I. karstenii*, of the ten enzymes scored, three (GDH, LAP and NSP) were represented by a single locus each and were monomorphic across all popu-

TABLE 1. Population designations, species, location of collection and voucher information for populations included in this study. The number in parentheses following the population designation indicates the number of individuals sampled from that population.

Population Designation	Species	Collection Location	Voucher & Location
S-4 (40)	*I. karstenii*	Merida, Venezuela	*Small 4 & Hickey,* MU
S-5 (40)	*I. karstenii*	Merida, Venezuela	*Small 5 & Hickey,* MU
S-6 (40)	*I. karstenii*	Merida, Venezuela	*Small 6 & Hickey,* MU
S-7 (40)	*I. karstenii*	Merida, Venezuela	*Small 7 & Hickey,* MU
S-8 (40)	*I. karstenii*	Merida, Venezuela	*Small 8 & Hickey,* MU
S-126 (20)	*I. karstenii*	Boyaca, Colombia	*Small 126 & Gonzalez,* MU, COL
S-127 (20)	*I. karstenii*	Boyaca, Colombia	*Small 127 & Gonzalez,* MU, COL
S-128 (20)	*I. karstenii*	Boyaca, Colombia	*Small 128 & Gonzalez,* MU, COL
			Small 129 & Gonzalez, MU, COL
S-141 (20)	*I. karstenii*	Boyaca, Colombia	*Small 141 & Gonzalez,* MU, COL
S-144 (20)	*I. karstenii*	Boyaca, Colombia	*Small 144 & Gonzalez,* MU, COL
J-79 (4)	*I. karstenii*	Cundinamarca, Colombia	*Jermy 17479,* BM
S-155 (20)	*I. palmeri*	Cundinamarca, Colombia	*Small 155 & Gonzalez,* MU, COL
			Small 156 & Gonzalez, MU, COL
			Small 157 & Gonzalez, MU, COL
J-77 (28)	*I. palmeri*	Cundinamarca, Colombia	*Jermy 17477,* BM
			Jermy 17478, BM
			Jermy 17480, BM
			Jermy 17481, BM
			Jermy 17482, BM

lations. The remaining seven enzymes, scored for nine putative loci, showed variation in at least one population. Several populations contained unique alleles in low frequencies, but no fixed, unique alleles were observed in any population. Allele frequencies for all populations are shown in Table 2 and a pairwise distance matrix (Nei's [1978] unbiased genetic distance) is shown in Table 3. Characterization of each polymorphic locus scored is described below. Population designations follow Table 1.

IDH.—This enzyme was coded by a single locus. All populations were monomorphic for allele *b* at this locus except for S-127 and S-128. The populations S-127 and S-128 expressed alleles *a* and *b,* with *a* being the most common (frequency 0.775) in S-127, and *b* being the most common (frequency 0.925) in S-128.

MDH.—This enzyme exhibited an exceedingly complicated banding pattern. Two putative loci were scored for this enzyme. The first was represented by a group of the most anodally migrating bands. These bands always comigrated in a constant pattern and showed additivity in putative heterozygotes. Two alleles were detected at this locus with allele *a* being the most common in all populations. Alleles *a* and *b* were present in all Venezuelan populations (except S-6, which was fixed for *a*) whereas all Colombian populations were monomorphic for allele *a*. The second locus scored consisted of a group of more cathodally migrating bands. This locus was uninterpretable in regard to

TABLE 2. Estimated allele frequencies for all populations sampled. Population designations and sample sizes follow Table 1.

	Population												
Locus	S-4	S-5	S-6	S-7	S-8	S-126	S-127	S-128	S-141	S-144	J-79	S-155	J-77
Gdh													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh													
a							0.775	0.075				0.500	0.446
b	1.000	1.000	1.000	1.000	1.000	1.000	0.225	0.925	1.000	1.000	1.000	0.325	0.250
c												0.175	0.304
Lap													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1													
a	0.875	0.975	1.000	0.550	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	0.125	0.025		0.450	0.100								
Mdh-2													
a	0.950	1.000	1.000	1.000	1.000								
b	0.050					1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgdh													
a	0.925	0.950	1.000	0.700	0.475	1.000	1.000	0.925	0.350	1.000			
b	0.075	0.050		0.300	0.525			0.075	0.650				0.304
c											1.000	1.000	0.696
Pgi													
a						0.425	0.600	0.075				0.500	1.000
b	1.000	1.000	1.000	1.000	1.000	0.550	0.400	0.925	1.000	1.000	1.000	0.500	
c						0.025							
Pgm													
a	0.100	0.725	0.850	0.184	0.363	1.000	1.000	0.925	0.725	0.500	0.500	0.500	0.571
b	0.900	0.275	0.150	0.816	0.367			0.075	0.275	0.500		0.400	
c											0.500	0.100	0.429
Skdh													
a								0.075					
b	1.000	0.837	1.000	1.000	1.000	0.500	0.475	0.400	0.475	0.300	1.000	0.500	0.625
c		0.162				0.500	0.525	0.525	0.525	0.700		0.500	0.375
Tpi-1													
a						0.025						0.071	
b	1.000	1.000	1.000	0.837	0.975	0.975	1.000	0.975	1.000	1.000	1.000	0.929	1.000
c				0.163	0.025			0.025					
Tpi-2													
a											0.125		
b	0.050					1.000	1.000	0.925	0.650	0.500	0.875	0.500	0.464
c	0.712	0.800	0.837	0.600	0.538			0.075	0.350	0.500			
d	0.238	0.200	0.163	0.400	0.463							0.500	0.536
Nsp													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

allelic variation, due to a complicated multi-banded pattern that was present in some individuals, but not in others. It was therefore scored conservatively as present or absent ("allele" *a* = present, "allele" *b* = absent). This locus was present in all individuals of all Venezuelan populations (with the exception

TABLE 3. Table of Nei's (1978) unbiased genetic distance for all populations sampled. Population designations follow Table 1.

Population	S-4	S-5	S-6	S-7	S-8	S-126	S-127	S-128	S-141	S-144	J-79	S-155
S-4	—											
S-5	0.028	—										
S-6	0.053	0.009	—									
S-7	0.032	0.066	0.094	—								
S-8	0.029	0.033	0.048	0.017	—							
S-126	0.322	0.243	0.239	0.395	0.313	—						
S-127	0.405	0.317	0.313	0.493	0.399	0.055	—					
S-128	0.203	0.123	0.128	0.261	0.201	0.107	0.158	—				
S-141	0.322	0.264	0.279	0.408	0.342	0.039	0.106	0.120	—			
S-144	0.283	0.250	0.280	0.372	0.322	0.070	0.139	0.119	0.004	—		
J-79	0.459	0.438	0.422	0.486	0.414	0.166	0.249	0.325	0.156	0.178	—	
S-155	0.395	0.371	0.401	0.414	0.349	0.191	0.158	0.241	0.199	0.204	0.114	—
J-77	0.531	0.477	0.482	0.525	0.436	0.206	0.180	0.310	0.172	0.190	0.080	0.050

of two individuals in S-4) and absent from all individuals of all Colombian populations.

6-PGDH.—This enzyme was scored for one locus. An additional zone of activity was observed, but not consistently, and so was not scored. Three alleles were detected at this locus; allele *a* was observed in the highest frequency in all populations except S-8, S-141 and J-79. Populations S-8 and S-141 had allele *b* as the most common allele, but J-79 was the only *I. karstenii* population to exhibit allele *c*.

PGI.—This enzyme was scored for one locus. Again, a second zone of banding was detected, but inconsistently, so was not scored. Three alleles were detected at this locus. Populations S-4 through S-8 (Venezuelan populations) were monomorphic for allele *b*. The Colombian populations all exhibited both alleles *a* and *b* or were fixed for allele *a*, except that S-126 had allele *c* in low frequency (0.025) in addition to alleles *a* and *b*.

PGM.—This enzyme was represented by a single locus with three alleles detected. In populations S-4 through S-144 only alleles *a* and/or *b* were detected in varying combinations. Allele *c* was detected only in population J-79 in conjunction with allele *a*.

SKDH.—This enzyme was represented by a single locus with three alleles detected. All populations were either fixed for allele *b* or had a combination of alleles *b* and *c*, except that S-128 exhibited allele *a* at low frequency (0.075).

TPI.—This enzyme was represented by two loci. A third zone of banding representing post-translational modification of locus two (Hickey et al., 1989a) was also observed. *Tpi-1* was relatively uniform across all populations. Allele *b* was by far the most common allele; three populations (S-7, S-8, and S-128) also exhibited allele *c* in low frequency, and one population (S-126) exhibited

allele *a* in low frequency. *Tpi-2* was a highly variable locus, exhibiting the highest number of alleles (four) in this study. All populations exhibited at least two alleles at this locus except that S-126 and S-127 were fixed for allele *b*.

In addition to the populations of *I. karstenii* surveyed, two populations of *I. palmeri,* a closely related tetraploid (Small, 1994) also were surveyed. These collections were made by two different collectors (R. Small and A. C. Jermy) in different years at the same locality (vicinity of Laguna Chisaca, Dpto. Cundinamarca, Colombia), which consists of a large lake and many surrounding pools. The S-155 and J-77 populations each consist of multiple collections from different sites within a single pool; the two populations, however, are clearly from different pools (A. C. Jermy, pers. comm.).

Isoëtes palmeri, like *I. karstenii,* was also monomorphic for isozymes GDH, LAP, and NSP and in addition was monomorphic for allele *a* at *Mdh-1,* and for allele *b* at *Mdh-2* (absence of the locus). The remaining loci exhibited two or three alleles. One allele was detected that was unique to, but not fixed, in *I. palmeri* relative to *I. karstenii*: *Idh* allele *c,* frequency = 0.341. These populations exhibited patterns of apparent fixed heterozygosity at some loci, as might be expected in an allotetraploid (Werth, 1989). Interestingly, however, patterns of fixed heterozygosity were polymorphic within these populations. For example, in the population S-155, individuals were collected at three sites within a pool of approximately 100 m^2 (corresponding to *Small & Gonzalez 155, 156* & *157* of Table 1). At the locus *Skdh,* all individuals showed the *bc* heterozygous phenotype; for *Idh,* however, the subpopulations 155 and 156 had an *ab* heterozygous phenotype, whereas the subpopulation 157 showed an *ac* heterozygous phenotype. Similarly, at *Pgm* subpopulations 155 and 156 exhibited an *ab* phenotype, whereas subpopulation 157 had an *abc* phenotype. Similar observations were made for the Jermy collections.

Measures of genetic variation were also calculated. The mean number of alleles per locus for *I. karstenii* was 2.3; for *I. palmeri* 1.7. The percentage of polymorphic loci for *I. karstenii* was 75%; for *I. palmeri* 50%. A matrix of Nei's (1978) unbiased genetic distance is shown for all populations in Table 3. Cluster analysis using UPGMA was performed on this matrix and the resulting phenogram is shown in Fig. 1. Finally, for *I. karstenii,* genetic distance was plotted against geographic distance between population groups, and a correlation coefficient was calculated (Fig. 2). There is a strong positive correlation between genetic and geographic distance with a correlation coefficient of r^2=0.775.

Mean F-statistics were also calculated for *I. karstenii*. The locus *Mdh-2* was removed prior to this analysis because it was scored on a presence/absence basis and therefore could not contribute to measures of heterozygosity. For *I. karstenii* the mean $F_{IS} = -0.060$, the mean $F_{IT} = 0.434$, and the mean $F_{ST} = 0.466$. These values suggest observations on the probable nature of genetic structure within *I. karstenii*. Given the relatively small negative value for F_{IS}, mating within subpopulations is probably near-random. This is not surprising, because *Isoëtes* is heterosporous, producing mega- and microgametophytes from separate spores, with fertilization accomplished by swimming sperm. In

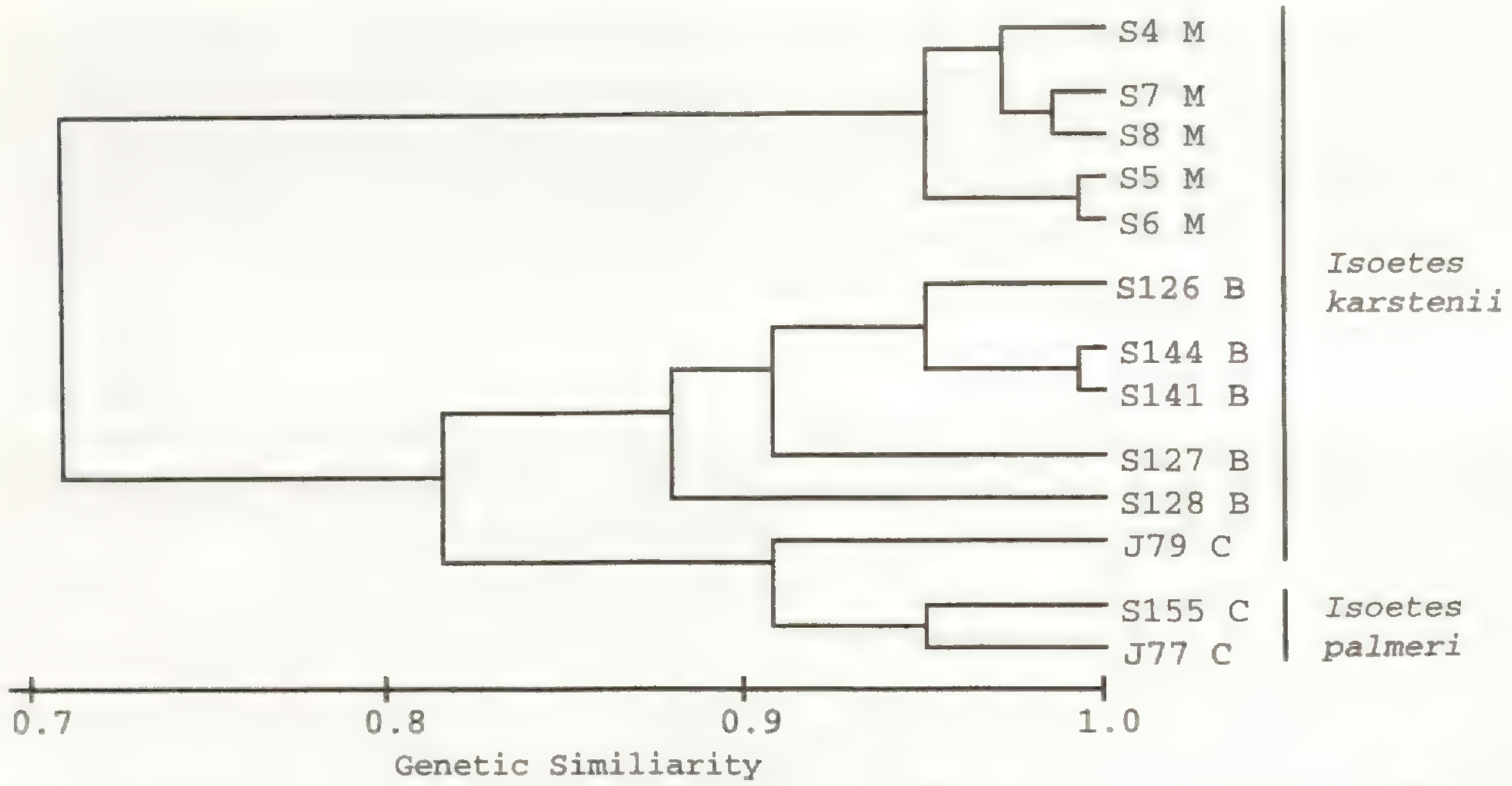

FIG. 1. UPGMA phenogram of population relationships based on Nei's (1978) unbiased genetic distance. Population designations follow Table 1; state of origin is indicated as "M" = Merida, Venezuela; "B" = Boyaca, Colombia; and "C" = Cundinamarca, Colombia.

addition, dispersal of spores is often accomplished via floating leaves that have become detached from the parent plant (or whole plants), yet still contain unbroken sporangia (pers. obs.). As the leaf decays, the spores are released, possibly relatively distant from the parental plant. On the other hand, the relatively high F_{ST} value suggests that mating among subpopulations is not random and that a high degree of differentiation is evident. This observation is further supported by the presence of many unique low-frequency alleles in single populations as well as the high correlation between genetic and geographic distance between populations.

DISCUSSION

Allozyme data have been used for many purposes in systematic studies. In *Isoëtes*, their most common uses have been to provide characters with which to distinguish species and to provide evidence regarding the parentage of allopolyploid taxa or hybrids (e.g. Taylor et al., 1985; Hickey et al., 1989b; Duff and Evans, 1992; Taylor and Hickey, 1992). Generally, fixed allelic differences between taxa at a given locus are used as evidence of reproductive isolation (biological species concept, Hickey et al., 1989b). Additive banding patterns in putative allopolyploid taxa or hybrids at a locus for which the putative parental taxa have fixed differences have been used to provide evidence of the parentage of the allopolyploid or hybrid (Taylor et al., 1985; Hickey et al., 1989b; Taylor and Hickey, 1992).

The results of the analyses of the populations of *I. karstenii* and *I. palmeri* revealed no unique, fixed alleles at any locus that can be used to differentiate these taxa. One locus (*Idh*) exhibited an allele that was unique to *I. palmeri*,

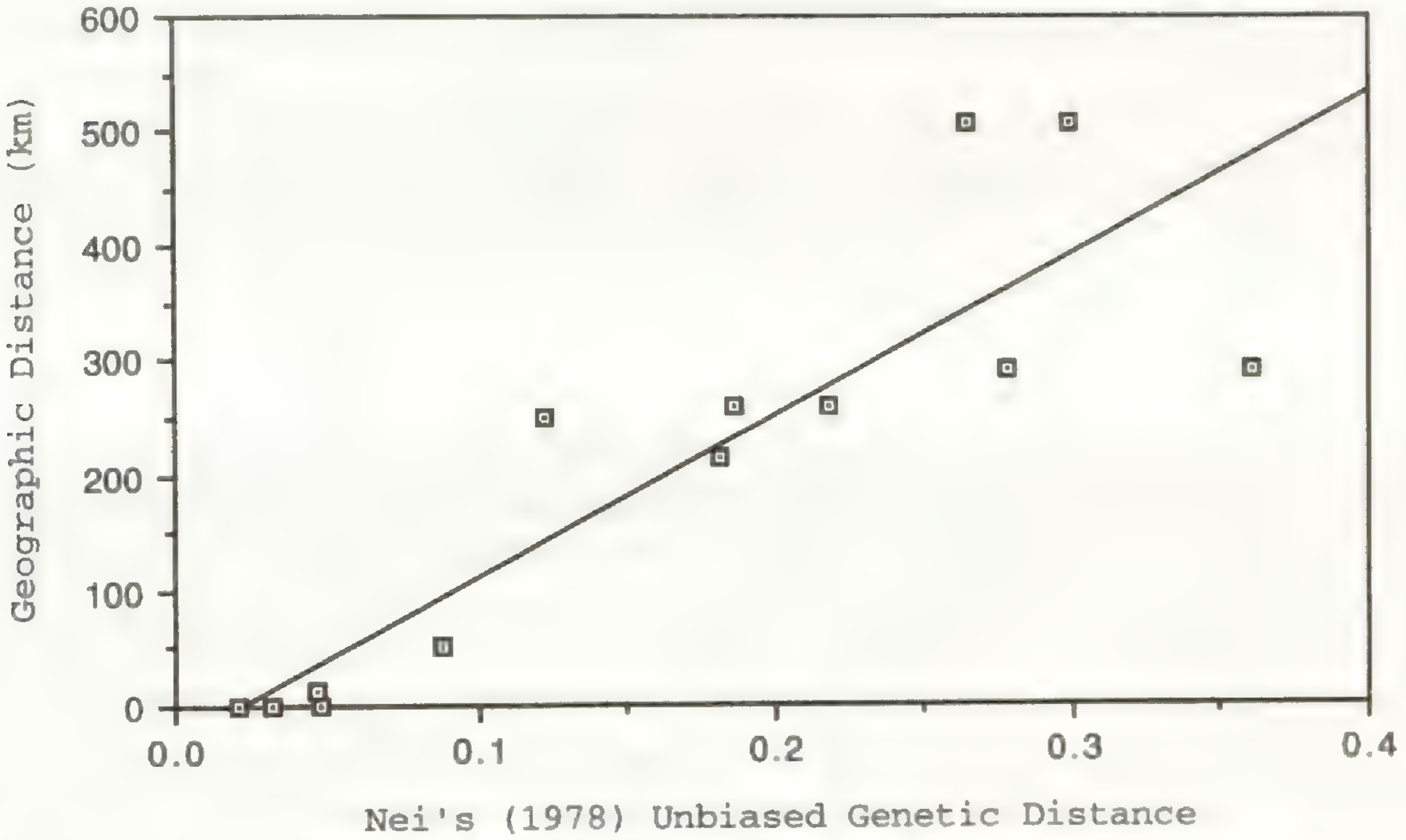

FIG. 2. Scatter plot of geographic vs. Nei's (1978) unbiased genetic distance between groups of populations of *I. karstenii* with line of best fit; $r^2=0.775$.

but not fixed. The fact that these two taxa are distinct species is strongly supported by morphological and cytological evidence (Small, 1994), but the allozyme data shed no light on this hypothesis. Several possibilities exist to explain the apparent lack of genetic divergence between *I. karstenii* and *I. palmeri*. First, this study incorporated a relatively small number of loci (12) and enzyme systems (10). Many authors have discussed the need to examine as many loci as possible (e.g. Archie et al., 1989), and Nei (1978) suggested that 50 or more loci should be examined. Secondly, the appearance of *Isoëtes* in the northern Andean páramos is relatively recent (early Pleistocene; van der Hammen and Cleef, 1986), which suggests that the speciation events that led to these taxa were probably also relatively recent and that there has been relatively little time for genetic divergence. Finally, *I. palmeri* is most likely an allotetraploid, with *I. karstenii* a probable parent (Small, 1994). Therefore half of *I. palmeri*'s nuclear genome is that of *I. karstenii,* and unless there were fixed, unique alleles contributed by the other parent, little or no genetic differentiation would be expected. It is interesting to note that the *I. palmeri* populations, both from Cundinamarca, Colombia, appear most closely related to the *I. karstenii* population that is also from Cundinamarca. Further sampling of *I. palmeri* from throughout its range is necessary to determine whether all *I. palmeri* populations are more closely related to the central Colombian populations of *I. karstenii* or whether multiple origins of the allotetraploid *I. palmeri* have occurred.

The values for genetic identity among populations determined in these analyses were compared with values previously presented in the literature (e.g., Gottlieb, 1977; Soltis and Soltis, 1989; Ranker, 1992). Conspecific populations of both homosporous pteridophytes and seed plants generally have very high

TABLE 4. Unbiased genetic identity (Nei, 1978) in *I. karstenii* by country of origin. N = number of populations sampled.

Country	N	Venezuela	Colombia
Venezuela	5	0.961	
Colombia	6	0.766	0.906

genetic identities (often I >0.95), although mean identity values as low as 0.78 have been reported for some ferns (Soltis and Soltis, 1989). Congeneric species of seed plants have relatively lower values (ca. 0.67 ± 0.07; Gottlieb, 1977), whereas congeneric species of homosporous pteridophytes exhibit an exceedingly wide range of genetic identities (0 to 0.85; Soltis and Soltis, 1989). The mean genetic identity of *I. karstenii* and *I. palmeri* (0.747) is comparable with the values for congeneric species. The genetic identity values among populations of *I. karstenii,* however, ranged from 0.611 to 0.996, with a mean identity of 0.811, a relatively low value that is nonetheless comparable with several homosporous pteridophytes (Soltis and Soltis, 1989). This can be explained, in part, by our sampling. Eleven populations of *I. karstenii* were sampled from a broad geographic range (Edo. Merida, Venezuela, to Depto. Cundinamarca, Colombia, a distance of ca. 600 km) and the resulting mean genetic identity was relatively low.

Furthermore, genetic identity between groups of populations of *I. karstenii* decreases as the distance between them increases. Tables 4 and 5 present mean genetic identity of populations of *I. karstenii* by country and state respectively. The mean identity among the Venezuelan populations, all collected in the state of Merida, was 0.961. Among the Colombian populations, collected in Deptos. Boyaca and Cundinamarca, which are separated by ca. 250 km, the mean genetic identity is lower with a value of 0.906. The mean genetic identity between these two countries is lower still, at 0.766. Further subdivision of the populations into the three states (Merida, Venezuela [northernmost], Boyaca, Colombia [central], and Cundinamarca, Colombia [southernmost]) reinforces the trend (see Table 5). Within the respective states, the mean genetic identity ranges from 0.932 to 0.961 (only a single population was available from Cundinamarca so a comparison could not be made). Genetic identity between Merida and Cundinamarca, the most widely separated locations, was the lowest (0.752), whereas genetic identity between Boyaca (central) and Merida (northern) and Boyaca and Cundinamarca (southern) were 0.769 and 0.855 respectively.

Similar results were found by Duff and Evans (1992) in the species pair *I. caroliniana* and *I. engelmannii.* Although *I. caroliniana* had a high mean interpopulational genetic identity (0.954), *I. engelmannii* had a relatively lower value (0.897). Duff and Evans (1992) cited several possible explanations for the lowered identity values in *I. engelmannii* including its age, low levels of interpopulational gene flow, and interspecific hybridization. Althought any of these factors may be responsible for the low genetic identity of the *I. karstenii*

TABLE 5. Unbiased genetic identity (Nei, 1978) in *I. karstenii* by state of origin. N = number of populations sampled.

State	N	Merida	Boyaca	Cundinamarca
Merida, Venezuela	5	0.961		
Boyaca, Colombia	5	0.769	0.932	
Cundinamarca, Colombia	1	0.752	0.855	no comparison

populations, it would appear that geographic distance and the resulting low levels of interpopulational gene flow have the strongest effects.

The F-statistics provide a measure of genetic differentiation within and between populations. Two conclusions can be drawn from these statistics: 1) intrapopulational genetic variation is high as evidenced by a slightly negative F_{IS}, and 2) interpopulational differentiation is also high as evidenced by the relatively high F_{ST}. That levels of gene flow between *Isoëtes* populations are low has long been assumed, based on levels and patterns of genetic variation (Hickey et al., 1989b; Duff and Evans, 1992; Taylor and Hickey, 1992), and the data presented here provide confirmation of this hypothesis, at least in the case of *I. karstenii*. These conclusions are further supported by data showing the high correlation between genetic and geographic distance (Fig. 2), and can be seen graphically in the phenogram where OTUs are also identified by state of origin (Fig. 1). This is, however, in contrast to the results found by Hickey et al. (1989b) for *I. storkii* T.C. Palmer. Geographic and genetic distance were not well correlated in this species, but probably resulted from local patterns of establishment and extinction of populations in a volatile volcanic and fire-prone habitat.

One final observation also indicates a high degree of interpopulational divergence. Within *I. karstenii* as a whole 75% of the loci are polymorphic, yet within any individual population there is a maximum of 58% polymorphic loci, with a mean of 33% polymorphic loci. This shows that loci that are polymorphic in one population may be monomorphic in another, further indicating population differentiation.

In addition to the calculations of genetic identity and population structure indices, allozymes may provide additional sources of information that can be useful in systematic studies. The occurrence of a post-translational modification product of the locus *Tpi-2* in *Isoëtes* was originally described by Hickey et al. (1989a). In addition to clarifying the interpretation of allozymes at this locus, this phenomenon provided a character of potential phylogenetic utility as it was observed in some, but not all species examined. Specifically, this character-state (presence of the post-translational modification) has been observed in *I. storkii* T.C. Palmer, *I. mexicana* Underw., *I. melanopoda* Gay & Durieu, *I. butleri* Engelm., *I. piedmontana* (N. Pfeiff.) C.F. Reed, *I. melanospora* Engelm., and *I. tegetiformans* Rury, but is absent in *I. cubana* Baker and *I. pallida* Hickey (Hickey et al., 1989a). This study adds two additional species that display this character state, *I. karstenii* and *I. palmeri*, and thereby extends the geographic distribution of this character state to northwestern South Amer-

ica. This serves as a potential link for these northern Andean species with some Central American/Mexican (*I. storkii, I. mexicana*) and North American (*I. melanopoda, I. butleri, I. piedmontana, I. melanospora, I. tegetiformans*) taxa while differentiating them from the Caribbean and southern Mexican species, *I. cubana* (Cuba) and *I. pallida* (Oaxaca, Mexico). Further sampling of this character may add significantly to our knowledge of biogeographic patterns and serve to unite currently fragmented species complexes.

The presence of patterns of apparent fixed heterozygosity in the tetraploid *I. palmeri* was expected, yet the polymorphism of fixed heterozygous patterns was not. Unfortunately, only two samples of *I. palmeri* were surveyed, both from the same general area; formal genetic tests to confirm the "fixation" of heterozygosity were not performed; and only one of the putative parents (*I. karstenii*; Small, 1994) was surveyed. Although hypotheses to explain this phenomenon might be presented (e.g., Gastony, 1990), our sampling is too limited to do so with confidence.

The data presented here confirm and extend results previously obtained regarding the distribution of genetic variation in populations of *Isoëtes* (Hickey et al., 1989b; Duff and Evans, 1992). F-statistics and analyses of geographic vs. genetic distance show that genetic variation in *Isoëtes* can be highly structured, most likely due to the paucity of interpopulational gene flow. Accordingly, these results show that, in studies of genetic variation in *Isoëtes,* it is imperative that sampling be performed from throughout the range in order to observe as much of the variation as possible. This will be especially important in analyses of allopolyploid species, given the possibility of multiple origins of those entities.

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REVIEW

Welsh Ferns, by G. Hutchinson and B. A. Thomas. 1996. Department of Botany, National Museum of Wales, Cathays Park, Cardiff, CF1-3NP, Wales. No ISBN. £11 (£13 for overseas orders, including postage and handling). vi, 265 pp., paperbound.

This attractive volume originally was published in 1940 as a field guide to Welsh ferns, and authored by H. A. Hyde and A. E. Wade. Updates and changes appeared through the fourth edition (1962). A fifth edition appeared in 1969, revised extensively by Wade's successor at the National Museum of Wales, S. G. Harrison, who also expanded the scope to include the fern allies. The coverage of fern hybrids was increased in Harrison's sixth edition (1978). With the continued refinement of the book through its lengthy printing history, one might ask what could be left to improve upon. The seventh edition, however, brings the treatment to new levels of detail and usefulness. Hutchinson and Thomas have reorganized the material from an outdated system of classification to a more practical, alphabetical enumeration of the genera within the ferns and fern allies, with the taxonomic sequence (following the second edition of *Flora Europaea*) summarized in the introduction for those readers interested in such matters. They also have expanded the species descriptions, added a short addendum on exotic ferns in Welsh gardens that might become naturalized in the future, and brought the citation of authorities into conformance with Brummitt and Powell's *Authors of Plant Names*. Species are mapped for Wales on a 10 km^2 grid over the country based on herbarium vouchers, and the overall European distributions are shown on shaded range maps. Thus, although the book has a similar superficial appearance to earlier editions, the contents have been improved substantially.

The 32 genera and 65 species of Welsh pteridophytes are separated using bracketed keys, which also include an additional 27 British species not yet recorded for Wales. Thus the book can be used elsewhere in the British Isles as well as in Wales. The largest genera are *Asplenium, Dryopteris,* and *Equisetum,* with nine, eight, and seven Welsh species, respectively, as well as a number of hybrids and infraspecific taxa. The keys and descriptions are eminently usable, with technical terms defined in an extensive glossary at the end and a detailed introduction to fern biology at the beginning. The drawings in the main portion of the book are nicely rendered and reproduced, although a supplementary series of drawings and black-and-white photographs at the end is of somewhat uneven quality. The lengthy discussions of hybrids and polyploidy make the book especially useful to field botanists. As an affordable field guide to a beautiful and naturally diverse portion of the British Isles, this title is recommended to both amateur and professional pteridologists.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

INFORMATION FOR AUTHORS

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David W. Bierhorst (1924–1997)

ALLAN WITZTUM
Department of Life Sciences, Ben Gurion University, P.O.B. 653, 84105 Beer Sheva, Israel

David W. Bierhorst passed away on May 19, 1997. He was Professor of botany at the University of Virginia (1953–1955), Cornell University (1955–1968), and the University of Massachusetts at Amherst (1968–1978). His interests in botany included the anatomy of vascular tissue and the morphology and evolution of ferns. Part of his original research is summarized in his richly illustrated text, "Morphology of Vascular Plants," which was published in the Macmillan Biology Series in 1971. His detailed studies of the anatomy and morphology of gametophytes and sporophytes of *Equisetum, Psilotum, Tmesipteris, Schizaea, Actinostachys,* and *Stromatopteris* are seminal contributions. He reexamined the nature of the leaf as an appendicular organ on the stem in the light of *Psilotum* and *Stromatopteris,* where sharp distinctions are absent. His conclusions made him wary of the idea that there is a close link between the family Psilotaceae and the very primitive fossil Rhyniales, and he believed *Psilotum* was more closely related to the extant ferns.

David William Bierhorst was born October 17, 1924, in New Orleans. He loved the natural history of the bayous and was an amateur herpetologist in his younger years. He served in the U.S. Marines in the Pacific theater during World War II and later returned to collect plants in Fiji (1962) and New Caledonia (1962, 1966). He collected plants in South America as well. He completed his Bachelors degree (1947) and Masters degree (1949) at Tulane University. He did his Ph.D dissertation with Professor E. C. Abbe at the University of Minnesota (1952).

When David Bierhorst was forced by heart disease to take early retirement from the University of Massachusetts, his beloved wife, Millie (whom he married in 1945), drove them south to Picayune, Mississippi where they lived for some years (1978–1986) before moving to New Iberia, Louisiana. David continued his interest in botany and grew ferns, *Lycopodium, Selaginella,* orchids and a vegetable garden. In his last ten years, he studied Hebrew, Aramaic, history, and archeology on his own and shared his new interests with his community, where he was regarded as a great teacher. He is survived by his wife Mildred Lambright Bierhorst, their daughter, Patricia Bierhorst Fischer, their son, Kenneth William Bierhorst, seven grandchildren, a sister, Edith Back, and a brother, Herman William Bierhorst.

I am privileged to count myself among his doctoral students (at Cornell), and both my wife and I have been "in touch" with him and his wife Millie during the 31 years since we left Cornell for Israel. He was a very special person. In a visit to David and Millie Bierhorst that my wife, Ann, and I made two years ago, we were witness to the courage of a man who faced a difficult illness with strength and grace, to the love in his family, and to the love of the community for him and for his wife. We are both grateful for having studied with David, for his friendship, and for that visit in New Iberia.

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American Fern Journal 87(4):120–126 (1997)

Interactive Effects of Polylactic Acid with Different Aluminum Concentrations on Growth, Pigment Concentrations, and Carbohydrate Accumulation of *Azolla*

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ABSTRACT.—The interactive effects of 1 μg l^{-1} polylactic acid (PLA) with aluminum concentrations of 2, 4, 8, and 16 mg l^{-1} on *Azolla caroliniana* growth, chlorophyll, carotenoid, anthocyanin, and carbohydrate accumulation were examined. *Azolla* growth was decreased with increases in aluminum concentration. Furthermore, the growth reduction was less pronounced when PLA was present. Chlorophyll *a* and *b* concentrations were decreased in plants grown at Al concentrations of 4 mg l^{-1} and higher. Polylactic acid reduced the influence of Al concentrations of 4, 8, and 16 mg l^{-1} on chlorophyll *a* and *b*. Similarly, carotenoid concentrations were decreased as Al concentrations increased in the growth media. Anthocyanin concentration was increased at 4.0 mg l^{-1} Al without PLA and at higher Al concentrations with or without PLA. In general, carbohydrate accumulation increased as Al concentration increased. However, this increase in carbohydrates was less pronounced in the presence of PLA. In the absence of Al from the growth media, treatment with 1 μg l^{-1} PLA resulted in an increase in plant growth, chlorophyll, and carotenoid concentration and a decrease in carbohydrate accumulation.

Aluminum (Al) toxicity is an important component of the damaging effects of acid rain in rivers and lakes. Rueter et al. (1987) reported that acid rain not only increased total soluble Al concentration, but also changed the affinity of organic and inorganic complexes with metals. Sprenger and McIntosh (1989) showed that Al concentrations were elevated in both water and submerged aquatic macrophytes (*Ranunculus ololeucos* J. Lloyd, *Luronium natans* Raf.) in acidified waters. According to Stanley (1974), an addition of 92 μmol l^{-1} Al to well-buffered medium inhibited 50% of the root growth of *Myriophyllum spicatum* L.

The toxicity of Al depends upon a number of factors, including pH and organic matter content (Tan and Binger, 1986). Aluminum toxicity increases at a pH below 5 because its solubility increases considerably. Of the different species of Al, Al^{3+} is the only significant one that is found in freshwater at a pH of 4 or less (Poleo, 1995). In general, Al has been shown to interfere with cell division, decrease respiration rates, interfere with enzymes governing the deposition of polysaccharide in cell walls, increase cell wall rigidity, and decrease the uptake and transport of water and several elements including Ca, Mg, P, and K in plants (Foy et al., 1978).

The earliest recorded use of lactic acid as a plant growth regulator was by

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Blumenthal and Meyer (1924), who observed callus formation from carrot slices exposed to lactic acid. In response to growth-inhibitory levels of heavy metals, plants synthesize metal-binding phytochelatins to detoxify excess metals (Kinnersley, 1993). Biostimulants such as polylactic acid have properties in common with phytochelatins and could be employed as a heavy metal detoxifier (Kinnersley, 1993). Aqueous lactic acid contains polymers of lactic acid which promote growth in some plants and unicellular algae (Kinnersley, 1993). This study was designed to evaluate the interactive effects of polylactic acid (1 $\mu g\ l^{-1}$) with different aluminum concentrations (2, 4, 8, and 16 $mg\ l^{-1}$) on *Azolla* growth, chlorophyll, carotenoid and anthocyanin content and carbohydrate concentration.

Materials and Methods

Cultures of *Azolla caroliniana* Willd. were grown in a diluted (1:40) Hoagland solution (Hoagland and Arnon, 1938) at pH 6.0. Plants were placed in a growth chamber with a 14 h photoperiod at 220 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density, and temperatures of 25 ± 1°C. A total of 2400 plants was selected arbitrarily and placed into 250 ml Erlenmeyer flasks (12 flasks per treatment). The flasks contained 125 ml diluted (1:40) Hoagland solution (pH of 3.8), and received 20 plants. The treatments were Al concentrations of 0, 2, 4, 8, and 16 $mg\ l^{-1}$ with and without the presence of 1 $\mu g\ l^{-1}$ dissolved polymer L-lactic acid. Polylactic acid was prepared following the method of Kinnersley et al. (1990). Aluminum was supplied in the form of $AlK(SO_4)_2.12H_2O$. The concentration of PLA was selected based on the earlier finding that the highest *Azolla* growth was obtained at PLA concentration of 1 $\mu g\ l^{-1}$ (Ayala-Silva and Al-Hamdani, 1997). *Azolla* was grown at the above conditions for 14 days and the growth medium was changed at the end of the first week. At the end of the second week of treatment, the plants from six randomly selected flasks of each treatment were used for dry weight and nonstructural carbohydrates measurements. The plants in the remaining six flasks of each treatment were used for chlorophyll *a* and *b,* carotenoid and anthocyanin determinations.

Dry Weight.—Samples were oven dried for 48 h at 70°C and the dry weight was recorded. The dried samples were then stored at −20°C for subsequent carbohydrate analysis.

Chlorophyll/Carotenoid Content.—Each sample (0.10 g fresh weight) was placed in a 10 ml vial (6 per treatment) containing 5 ml of N,N-dimethylformamide (N,N-DMF). The samples were stored in the dark at 4°C for 36 h and assayed spectrophotometrically following the procedures of Inskeep and Bloom (1985). Total carotenoid concentration was determined spectrophotometrically from the N,N-DMF extraction, and total concentration was calculated using the formula of Doong et al. (1993).

Anthocyanin Content.—Samples of 0.10 g fresh weight were homogenized in 5 ml of methanol containing 1% HCl (v/v). The homogenates were then

TABLE 1. Interactive effects of polylactic acid (PLA) concentration of 1 μg l^{-1} and different concentrations of aluminum (mg l^{-1}) on *Azolla* growth. LSD = Least Significant Difference value for difference between means within a column.

Treatment	Plant—Dry wt. (mg)
Control (without Al and PLA)	34.0
PLA	41.0
2.0 Al with PLA	30.0
2.0 Al without PLA	27.0
4.0 Al with PLA	25.0
4.0 Al without PLA	23.0
8.0 Al with PLA	25.0
8.0 Al without PLA	21.0
16.0 Al with PLA	17.0
16.0 Al without PLA	14.0
LSD (0.05)	4.0
LSD (0.01)	5.0

filtered and absorbance of the extracts determined spectrophotometrically by the method of Mancinelli (1990).

CARBOHYDRATE DETERMINATION.—Carbohydrate analysis of the plant samples was conducted following a procedure slightly modified from that outlined by Chatterton et al. (1987). Samples were ground into a fine powder and a 100–500 mg portion was placed in a sealed vial and used for the determination of soluble sugars, starch, and total nonstructural carbohydrates (TNC) as reported in detail by Wilson and Al-Hamdani (1997).

STATISTICAL ANALYSIS.—This experiment was repeated twice and analyzed statistically as a randomized complete block design (Steel and Torrie 1980). This design ensured that observed differences in plant performances were largely due to treatments rather than variation among blocks (experiment conducted at different times). Mean separations for the treatments that had significant F values ($P > 0.05$) in the ANOVA tests were on the basis of the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

After, 14 days, growth of *Azolla* was decreased as the aluminum concentrations were increased. The exception was that of 2 mg l^{-1} Al, which in the presence of PLA did not significantly decrease *Azolla* growth (Table 1). The highest growth reduction (58.8%) was documented for plants grown at 16.0 mg l^{-1} Al in the absence of PLA (Table 1). Aluminum was shown to interfere with cell division, decrease respiration rates, interfere with enzymes governing the deposition of polysaccharide in cell walls, increase cell wall rigidity, and decrease the uptake, transport, and use of water and several elements including Ca, Mg, P, and K in plants (Foy et al., 1978). The presence of PLA in the media had no significant influence on Al effects on plant growth at the ex-

TABLE 2. Interactive effects of polylactic acid (PLA) concentration of 1 μg l^{-1} and different concentrations of aluminum (mg l^{-1}) on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoid and anthocyanin concentration in *Azolla*. LSD = Least Significant Difference value for difference between means within a column.

Treatment	Chl *a* mg g^{-1} fr. wt	Chl *b* μg g^{-1} fr. wt	Carotenoid	Anthocyanin
Control (without Al and PLA)	7.53	5.49	72.39	2.00
PLA	8.32	6.15	81.69	2.84
2.0 Al with PLA	7.42	5.29	65.52	2.90
2.0 Al without PLA	7.40	5.14	62.08	2.93
4.0 Al with PLA	6.31	4.40	64.12	3.03
4.0 Al without PLA	5.81	3.98	63.81	3.72
8.0 Al with PLA	3.17	2.13	37.44	11.46
8.0 Al without PLA	2.47	1.34	35.40	11.90
16.0 Al with PLA	2.76	2.14	32.30	17.28
16.0 Al without PLA	2.35	1.42	30.33	19.57
LSD (0.05)	4.00	3.40	5.89	1.42
LSD (0.01)	5.30	4.60	7.89	1.90

amined Al concentrations (Table 1). However, in the absence of Al, PLA caused significant increase in *Azolla* growth. Furthermore, the reduction of growth as influenced by Al concentrations was less pronounced when PLA was present. Aqueous lactic acid containing polymers of lactic acid was reported to promote growth in a variety of plants and unicellular algae (Kinnersley, 1993). In earlier studies, Kinnersley et al. (1990) found that 1000 μg l^{-1} of the polymer L-lactic acid resulted in a significant increase plant biomass in duckweed (*Lemna minor* L.), an effect that was accompanied by an increase in chlorophyll accumulation.

Chlorophyll *a* and *b* concentrations were decreased in plants grown at Al concentration of 4 mg l^{-1} and higher (Table 2). In the absence of Al from the growth medium, PLA increased the concentration of chlorophylls *a* and *b* (Table 2). Similarly, the concentration of carotenoid in *Azolla* was increased by the presence of 1 μg l^{-1} PLA and decreased as Al concentrations increased in the growth media (Table 2).

The actual mechanism of action of polylactic acid is unknown. However, the increase in plant growth and chlorophyll concentration documented here for *Azolla* could be due to polylactic acid enhancing plant nutrient assimilation or utilization. Kinnersley et al. (1990) found that polylactic acid stimulated growth of *Lemna* in reduced nutrient media. On the other hand, the interactions of Al with mineral nutrients have been well-documented in many plant systems in the recent past. For example, Al has been shown to interfere with the uptake and accumulation of certain nutrients and the transport and use of essential elements, including N, P, K, Ca, Mg, Na, Fe, Cu, Mn, and Zn (Balakumar et al., 1991). In response to elevated levels of heavy metals, plants synthesize metal-binding phytochelatins to detoxify excess metals. Biostimulants such as polylactic acid have properties similar to phytochelatins and could be employed as a heavy metal detoxifiers (Kinnersley, 1993).

TABLE 3. Interactive effects of polylactic acid (PLA) concentration of μg l^{-1} and different concentrations of aluminum (mg l^{-1}) on carbohydrate concentration in *Azolla*. LSD = Least Significant Difference value for difference between means within a column.

Treatment	Sucrose	Starch	Total nonstructural carbohydrates
	(μg g^{-1} dry wt.)		
Control (without Al and PLA)	5.48	261.42	355.50
PLA	6.80	217.50	300.85
2.0 Al with PLA	7.17	292.78	395.37
2.0 Al without PLA	8.41	314.50	415.46
4.0 Al with PLA	9.51	322.13	414.20
4.0 Al without PLA	10.90	393.46	499.17
8.0 Al with PLA	11.72	362.00	469.70
8.0 Al without PLA	12.30	400.00	510.03
16.0 Al with PLA	13.10	477.00	590.97
16.0 Al without PLA	17.90	515.00	634.47
LSD (0.05)	1.38	37.46	39.58
LSD (0.01)	1.84	49.47	52.79

Anthocyanin concentration was increased at the Al concentration of 4.0 mg l^{-1} without the presence of PLA and at higher Al concentrations with or without PLA (Table 2). The highest increase in anthocyanin concentration (879%) was obtained for plants grown at 16 mg l^{-1} Al without PLA. Anthocyanin induction in *Azolla* has not been yet extensively studied. These measurements may provide an opportunity to relate its formation to biomass and chlorophyll formation. Many aquatic plants possess anthocyanins. These pigments are produced in response to various stress-related factors such as high light intensity, high temperatures or nutritional limitations (Doong et al., 1993). Haug (1984) suggested that anthocyanins are capable of chelating Al. This indicates that *Azolla* increased anthocyanin synthesis as mechanism of detoxifying Al.

Sucrose concentration of *Azolla* increased with the increase in Al concentration in the growth media (Table 3). However, sucrose concentration was not significantly different among the same Al concentrations when PLA was present. The exception was for plants grown at 16 mg l^{-1} Al, in which the concentration of sucrose was increased by 36.6% in comparison to that of the same concentration in the absence of PLA. Starch accumulation in *Azolla* was increased as Al concentrations were increased in the growth media (Table 3). However, this increase in starch accumulation was less distinct in the presence of PLA. In addition, PLA influenced reduction in starch accumulation in plants grown at Al concentrations greater than 2 mg l^{-1} in comparison to that obtained of plants grown at the same Al concentrations in the absence of PLA. Similar results were obtained for the TNC with the exception that in the absence of Al, PLA resulted in an decrease in total nonstructural carbohydrates (TNC) accumulation (Table 3). This should not be interpreted as Al causing an increase in carbohydrate synthesis. Rather, it most likely resulted from a reduction in carbohydrate utilization. An increase in carbohydrate accumulation in response to Al was also found to take place in *Salvinia minima* Baker

(Gardner and Al-Hamdani, 1997). Schroll (1978) observed that metal toxicity inhibited photosynthesis less than overall plant growth. Furthermore, Roy et al. (1988) reported that Al decreased respiration and carbohydrate utilization. Lower rates of carbon assimilation and a decrease in yield are associated with carbohydrate accumulation in several plant species (Azcon-Bieto 1983). It seems likely, therefore, that Al caused an increase in carbohydrate accumulation as plant growth was decreased.

In conclusion, this study has shown that Al is toxic to *Azolla* plants when provided at concentrations higher than 2 mg l^{-1}. In most cases, polylactic acid reduced the damaging effects of aluminum. This may be due to binding of polylactic acid with Al, resulting in a lower effective Al concentration within plant cells.

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New Records of Pteridophytes for Botswana

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ABSTRACT.—In a survey of the pteridophytes of Botswana, the family Isoetaceae was encountered and is a new record for Botswana. The family is represented by a single species, *Isoetes schweinfurthii*. Also recorded for first time in Botswana were three other species: *Ophioglossum lancifolium, Actinopteris dimorpha,* and *Marsilea minuta.*

Although the pteridophytes of southern Africa have been thoroughly and very capably surveyed (Schelpe, 1970; Jacobsen, 1983; Schelpe and Anthony 1986; Burrows, 1990), new explorations are adding to knowledge regarding their specific ranges. Botswana is a semi-arid country situated in the south-central part of the African continent and has three major ecological regions (Parson, 1993). In the northern region there are the Okavango Delta and the ancient lake beds of the Makgadikgali Pans. The southern area comprises the Kalahari Desert, characterized by fossil sand dunes and scrub and bush savanna. Between the Okavango-Makgadikgali and the Kalahari Desert, is an intermediate ecological zone known as the Hardveld region, dominated by sandy soils.

The records for ferns and fern allies in Botswana have yet to be finalized. Herein we report the occurrence of four pteridophyte species not yet recorded for the region (Fig. 1). One of them is a representative of a family also new to the records for Botswana.

RESULTS

The family Isoetaceae contains nine species in South Africa distributed in all countries of the region with the exception of Botswana, according to Burrows (1990). In our survey of the pteridophytes of Botswana, we found *Isoetes schweinfurthii* at a single locality. Other new records include *Ophioglossum lancifolium, Actinopteris dimorpha,* and *Marsilea minuta.* Details are presented below and localities are mapped in Fig. 1.

Isoetes schweinfurthii A. Braun in Baker.

Rhizomorphs are trilobed, up to 15 mm in diameter, and have scales that are horny, dark-brown, and deltate to elongate. Each plant may have up to 40 erect sporophylls in number, 11–38 cm in length, with persistent orbicular

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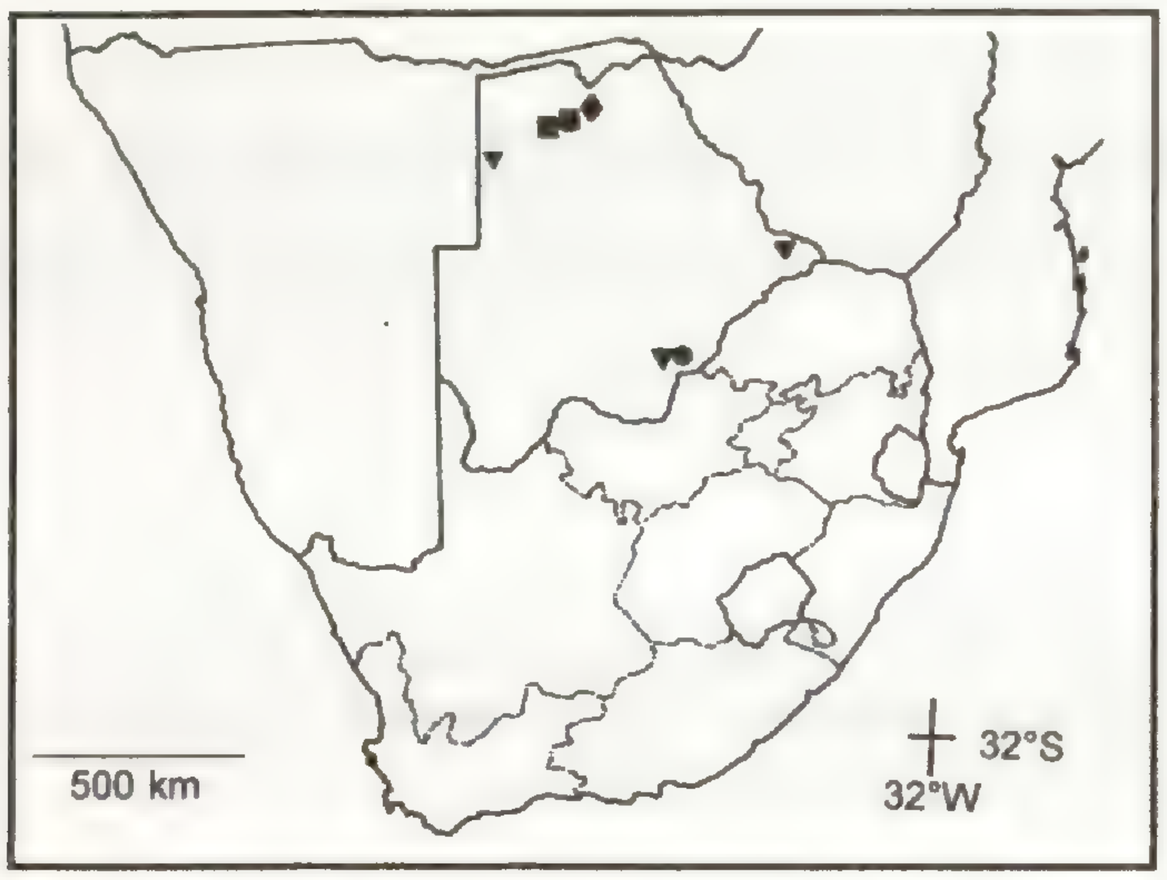

FIG. 1. Geographical distribution of new pteridophyte records from Botswana (● = *Isoetes schweinfurthii*, ◆ = *Ophioglossum lancifolium*, ▼ = *Actinopteris dimorpha*, ■ = *Marsilea minuta*).

bases 5–10 mm broad with tapered apices and deltate ligules. The sporangia are obovate to orbicular, 7–12 mm × 6–8 mm, without a velum. Megaspores have prominent tubercles on their distal faces and are flat or tuberculate on proximal faces. Microspores are alate.

Isoetes schweinfurthii is the only species of this family occurring in Botswana, having been localized in the southeastern section of the country, north of Kgale Siding near the Gaborone Dam. Its general distribution includes the areas of eastern Transvaal in South Africa, Namibia, Zimbabwe, Angola, Mozambique, Tanzania, Sudan, Central Africa Republic, Ivory Coast, Senegal, Moroco and Madagascar. Its specific habitats are pools in rocky outcrops. Herbarium voucher: *Hansen 3401* (PRE).

Ophioglossum lancifolium C. Presl.

Rhizomes are elongate, up to 5 cm in length. Roots are thick and horizontal. Vegetative blades are linear to oblanceolate, or narrowly elliptic, 15–80 mm × 2–12 mm. Bases are cuneate, gradually tapering into an elongate pseudo-petiole, and the apices are apiculate. Fertile blades are 30–150 mm in length and are inserted at the base of a sterile blade. Sporangia are in 8–23 pairs.

Ophioglossum lancifolium is distinguished from two other species of the genus occurring in Botswana by morphology of the vegetative blade and of the rhizome. Vegetative blades are narrowly elliptic to linear-lanceolate (less than 12 mm broad) in *O. lancifolium,* and elliptic to ovate-cordate (up to 40 mm broad) in *O. polyphyllum* A. Braun. *Ophioglossum lancifolium,* with oval to linear rhizomes, can be distinguished from *O. costatum* R. Br., which has orbicular rhizomes.

Ophioglossum lancifolium is found in seasonally wet places in the region of Savuti, north of Leopard Hills in North Botswana. It is generally distributed in the southwestern portion of the Cape Region of South Africa and scattered

in eastern South Africa to Zimbabwe, Namibia, Angola, Zambia, Malawi, Tanzania, Kenya, Uganda, Zaire, Mali, Ethiopia, Madagascar, Réunion and the Comoro Island. Herbarium voucher: *Jacobsen 3523* (PRE).

Actinopteris dimorpha Pic. Serm.

Rhizomes are 5 mm in diameter, with pale brown scales up to 5 mm long. Fronds are densely tufted, erect, coriaceous, and dimorphous. The stipes are glaucous, mostly glabrous, bearing a few linear scales up to 19 mm long. Blades are flabellate with truncate bases, and dichotomously divided into as many as 16 glaucous, linear segments. Fertile fronds are longer than vegetative ones. Segments are entire, but with 5–7 teeth at the apex. Margins usually are reflexed, with the undersurface having linear brown scales.

Actinopteris dimorpha can be distinguished from the one other related species of the genus occurring in Botswana, *A. radiata* (Sw.) C. Chr., primarily by its scale morphology and number of blade segments. *Actinopteris dimorpha* bears a few linear scales, whereas those of *A. radiata* are lanceolate. *Actinopteris dimorpha* has up to 16 glaucous, linear segments, whereas *A. radiata* has 20–48 segments.

Actinopteris dimorpha has been collected in the Aedume Park in Gaborone (*Hansen 3350,* PRE), in the Lepoloke Hills north of Bobonong (*Campbell 179.* PRE), in the Aha Hills of the Ngamiland District (*Long and Rae 374,* PRE), and in the Kgale Montain in the vicinity of Gaborone (*Mott 1015,* PRE). It is distributed in the Transvaal Region of South Africa, Zimbabwe, Mozambique, Malawi, Zambia, Tanzania, Somalia, Madagascar, the Comoro Islands, and Réunion. The habitat is generally rock crevices and boulders.

Marsilea minuta L.

Fronds of this species have leaflets thinly pilose, usually glabrous and not translucent. Leaflets of the floating forms are ovate with distal margins rounded, entire or shallowly irregular. Stipes of the dry form are up to 13 cm in length, the leaflets obovate, and distal margins entire or sinuate. There are (1)2–4 sporocarps per cluster, these densely pilose and becoming glabrous with age. Sporocarps are variable in size, elliptic in dorsiventral cross-section, dark brown to almost black, and both teeth are very prominent.

Marsilea minuta is distinguished from other closely related species of the genus in Botswana primarily by the morphology of its pedicels and sporocarps. Pedicels in *M. minuta* are branched, separating it from eight other non-branched species ocurring in Botswana. *Marsilea minuta* also has 2–4 sporocarps per cluster with prominent teeth, separating it from *M. apposita* Launert (inferior tooth absent) and *M. ephippiocarpa* Alston (3–10 sporocarps per cluster).

In Botswana, *M. minuta* has been collected on Xhere Island (*Smith 652,* PRE) and the Thaoge River (*Smith 1525,* PRE) in the Okavango Delta. It is generally distributed in Natal South Africa, Mozambique, Zambia, Malawi, Angola,

widespread through Africa to Guinea Bissau and Algeria, Madagascar, the Comoro Islands, and India. The habitat is in areas that are seasonally flooded.

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Gametophyte Development and Antheridiogen Activity in *Thelypteris ovata* var. *lindheimeri*

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ABSTRACT.—Gametophyte development was studied in *Thelypteris ovata* var. *lindheimeri* from spore germination to 48 days. Spores were sown aseptically onto the surface of agar-solidified mineral medium in multispore cultures. Germination began four days after spore sowing. Gametophytes were spatulate in morphology at six days and terminal meristems were initiated at ten days. Antheridia were observed at 19 days, archegonia at 21 days. At 28 days, gametophytes were antheridiate (smaller, 0.6–1.0 mm in width), archegoniate (larger, 1.3–2.3 mm in width) or asexual (variable, 0.5–1.5 mm in width). Gametophytes remained unisexual at 48 days. The presence of an antheridiogen system was investigated using culture media extracts of *T. ovata* gametophytes. Young gametophytes of *T. ovata* and *Onoclea sensibilis* L. form antheridia prematurely when exposed to partially purified culture media extracts. Two bands of biological activity were present after thin-layer chromatography, indicating the presence of at least two biologically active compounds.

Studies of gametophyte growth and development play an important role in identifying new species of ferns with antheridiogen systems. Antheridiogens influence growth and development of gametophytes and their sexual morphology. They are synthesized by fern gametophytes and cause premature antheridia formation in developing gametophytes. Antheridiogen systems have been described in several genera of ferns, including *Anemia, Ceratopteris, Lygodium, Onoclea,* and *Pteridium* (reviews by Näf et al., 1975; Raghavan, 1989). In the schizaeaceous ferns, *Anemia* and *Lygodium,* antheridiogens also can substitute for the light requirement for spore germination. Morphological characteristics of fern gametophytes have been suggested to be of value in the classification of some fern groups (Stokey, 1951; Atkinson and Stokey, 1964). These morphological characteristics are obtained by studies involving growth and development of fern gametophytes from spore germination to reproductive maturity. Unfortunately, these studies are few considering the number of fern species (approximately 10,000 species; Bold et al., 1987). Differences between ferns concerning spore germination and prothallial development have been determined (Nayar and Kaur, 1971). The influence of environmental conditions such as light intensity and quality on gametophyte development in a limited number of species has also been investigated (review by Raghavan, 1989).

This study describes gametophyte development in *Thelypteris ovata* R.P. St. John var. *lindheimeri* (C. Chr.) A.R. Sm. from spore germination to mature sexual gametophytes at 48 days after spore sowing. Little detailed research has been done concerning gametophyte development in this species. *Thelypteris ovata* is native to Mexico, the Caribbean Islands, and the southeastern U.S. west to central and southwestern Texas. It often grows in wet soils along riv-

erbanks (Lellinger, 1985) and also is cultivated as an ornamental (personal observation). The var. *lindheimeri* is commonly called Lindheimer's maiden fern.

Partial purification of an antheridiogen in *T. ovata* var. *lindheimeri* was undertaken after preliminary studies in our laboratory indicated the presence of an antheridiogen system. The presence of an antheridiogen in *Thelypteris hexagonoptera* (Michx.) Weath. was demonstrated using *Onoclea sensibilis* L. as the bioassay organism, and *T. hexagonoptera* and *Thelypteris* sp. (Dryopteroids) have been shown to be sensitive to the antheridiogen of *Pteridium aquilinum* (L.) Kuhn (Näf, 1956); however, few details were provided in that study. The present study is the first to report an antheridiogen system in *T. ovata*. *Onoclea sensibilis* also is shown to be sensitive to the *T. ovata* antheridiogen.

Materials and Methods

Spores of *T. ovata* var. *lindheimeri* (referred to hereafter as *T. ovata*) were collected from D'Hanis, in Medina County Texas, USA, May, 1995, and stored in glass vials at 4°C until used. Good spore viability was obtained with this storage temperature. A voucher specimen collected by J. Nester-Hudson was deposited in the Warner Herbarium, Sam Houston State University, Huntsville, Texas.

Spores were surface sterilized with calcium hypochlorite (1%, w/v) using aseptic procedures as described previously (Schedlbauer, 1976), except that the overnight soak was omitted. Spores were then sown onto the surface of 0.6 % (w/v) agar-solidified mineral medium containing Parker's macronutrients and Thompson's micronutrient (Klekowski, 1969) with the addition of one drop of 1% ferrous chloride per liter instead of the Fe-EDTA. Gametophytes were grown in sterile plastic Petri plates (100 × 15 mm) under fluorescent light on a 16-hr light (approximately 150 lumens/ft_2), 8-hr dark cycle at 20–24°C.

For studies of growth and development, microscopic slides were prepared of 15–30 gametophytes every 2–3 days for 48 days. Gametophytes were fixed with Hoyer's mountant/fixative and stained with 0.5% acid fuchsin. Gametophytes on these slides were photographed using a Nikon photomicroscope with Kodak T-MAX 100 film. Gametophyte size was determined from prepared slides using an ocular micrometer and compound microscope. This study was replicated three times and similar results were obtained in each study.

In another study, individual gametophyte growth and development were monitored for 28 days to determine the time of spore germination, meristem formation and reproductive morphology at 28 days for at least 200 gametophytes. Spores were sterilized and sown as described above. In order to monitor germination and growth of individual gametophytes, a 2 cm^2 square of black plastic screening was taped to the underside of at least 5 plates and ungerminated spores in the screen grid were plotted on a separate sheet of paper. The screening facilitated the plotting of ungerminated spores and aided in monitoring individual gametophyte growth. Spores and subsequent game-

tophytes were observed daily for 28 days after spore sowing. After 28 days, most cultures were too crowded for further observations. This study also was repeated three times with similar results each time.

Scanning electron micrographs of spores were obtained with a JSM 330A scanning electron microscope. Spores were mounted onto double-stick tape, sputter coated with gold palladium, and viewed at 15 kV.

For studies of antheridiogen activity, spores of *T. ovata* were sown aseptically onto the surface of agar-solidified mineral medium in 100 × 15 mm plastic Petri plates and placed under fluorescent lights. Eighteen plates were staggered in three layers in aluminum pans, allowing a large number of plates with gametophytes to be grown in a limited space. No visual growth differences between the bottom and top layers could be observed. After two months, gametophyte culture media were frozen and then thawed to obtain an aqueous extract. This extract was adjusted to pH 2.5 with 2 M HCl and partitioned 3× with ½ volume of ethyl acetate. The ethyl acetate extract was dried with $MgSO_4$, filtered through Whatman No. 1 filter paper, and evaporated to near dryness. One liter media equivalents were placed in silylated glass vials (5% dimethydichlorosilane in toluene), evaporated to dryness and stored at -20°C. The residue (equivalent to one liter) was dissolved in ethanol and applied to a 20 mm^2 glass-backed thin-layer chromatography (TLC) plate (silica gel 60A, 0.25 mm thick, Whatman International Ltd. Maidstone, England) and chromatographed 15 cm in ethyl acetate:chloroform:acetic acid (75:25:5; v/v/v). Plates were divided into ten equal strips and compounds were eluted from the silica gel with methanol. These fractions were placed in silylated glass vials, evaporated, and stored at −20°C for subsequent analysis by bioassay.

For bioassays of antheridiogen activity involving the premature formation of antheridia by gametophytes of *T. ovata* and *O. sensibilis,* fractions after TLC were dissolved in ethanol and a portion equivalent to 300 ml original culture media was added to 15 ml liquid culture medium that was buffered with 25 mM MES (2-[N-Morpholino]ethanesulfonic acid) at pH 6.0. This bioassay media also was diluted 50%. *Onoclea sensibilis* spores were collected on the grounds of Sam Houston State University in Dec. 1995 and stored at -20°C until used. These spores were rinsed several times in sterile water before sowing and no sterilant was used. Liquid mineral media with a portion of TLC fractions were placed in 35 x 10 mm sterile plastic Petri plates in which a piece of Millipore filter (type HA, 0.045 mm) was placed on top of a ring of Tygon tubing (2 mm thick × 1.5 mm diameter). A sufficient quantity of liquid media (approx. 2 ml) was added to each plate to wet the filter paper. New spores of *T. ovata* and *O. sensibilis* were sown onto the surface of the filter paper in replicate cultures. This bioassay arrangement allowed gametophytes to grow on a firm surface, yet be assayed with the convenience of liquid culture media. These bioassays were placed under florescent lights. Microscope slides were made of gametophytes after 14 days for *O. sensibilis* and after 17 days for *T. ovata* for observations of the formation of premature antheridia. This experiment was repeated at least three times.

In a separate bioassay, *T. ovata* and *O. sensibilis* spores were sown on liquid

media with TLC extracts and placed in darkness to test for induction of dark germination of spores. Cultures placed in darkness were checked at 7 days for germination (since most germination had occurred in control cultures by 7 days) and then placed in the light for an additional 10 days for *O. sensibilis* and 14 days for *T. ovata* for observations of premature antheridia formation. This experiment was repeated three times.

Gametophytes of *T. ovata* also were bioassayed with gibberellic acid (GA_3). A dilution series of 1 mM, 0.1 mM, 0.01 mM, 0.001 mM, and 0.0001 mM GA_3 was bioassayed as described above and repeated three times. Bioassays were placed in the light and darkness. Microscopic observations of spore germination in darkness were made eight days after sowing. Microscope slides were made after 14 days for detection of antheridia formation in *T. ovata* of bioassays in the light.

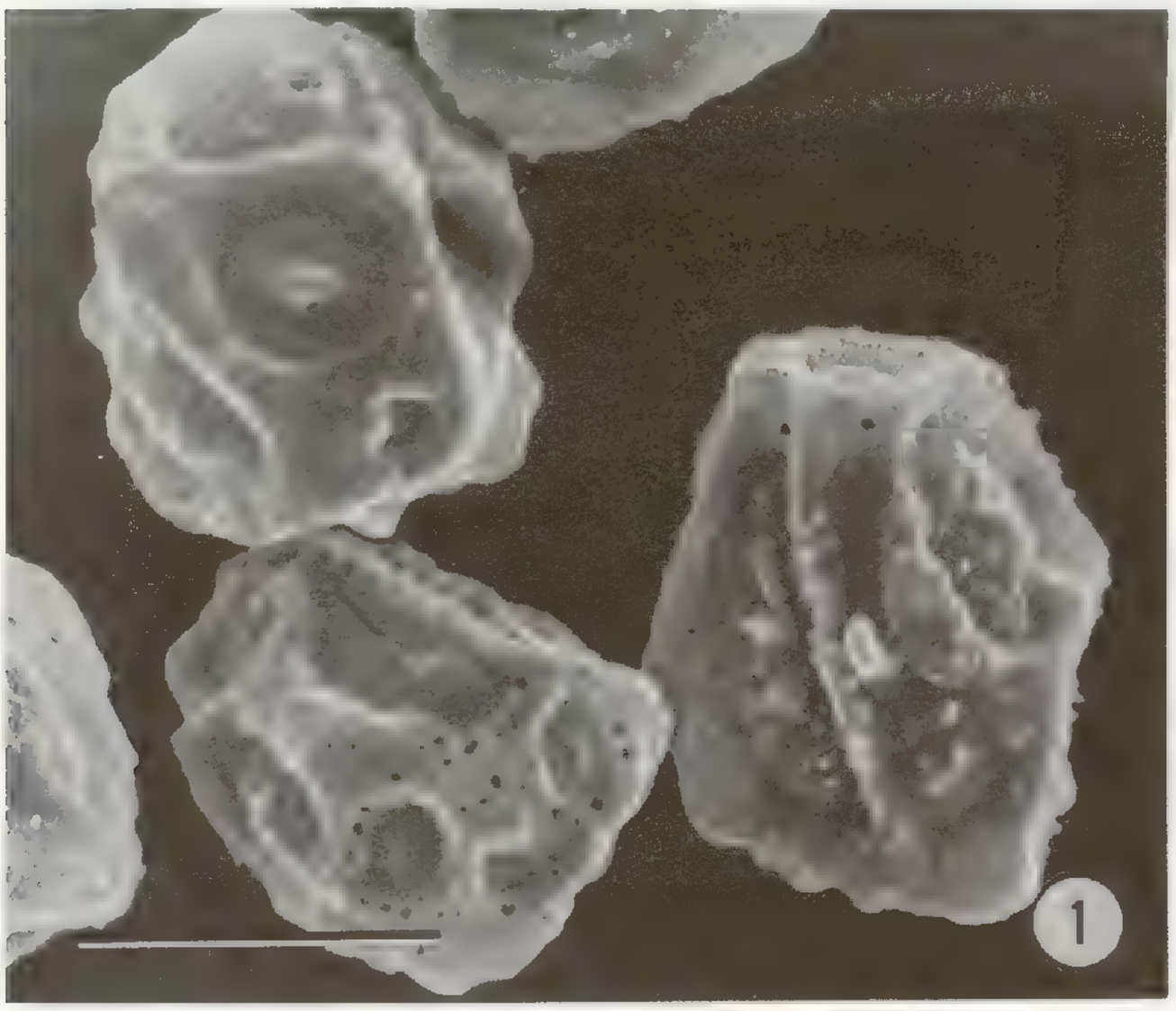
Results

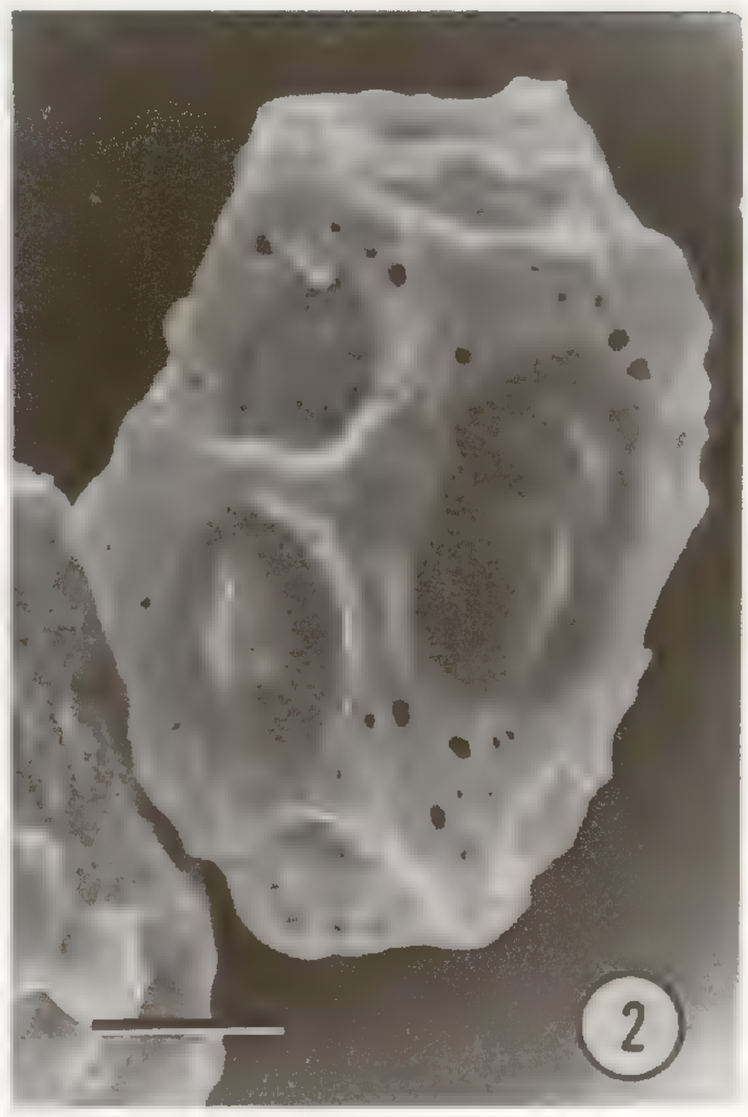
Spores of *T. ovata* are monolete, approximately 45 μm long × 30 μm wide, and have continuous connected folds that are 2–8 μm wide (Figs. 1, 2). The pattern of the folds varies, and folds may or may not have small perforations that vary in size. In mass, they are black in color.

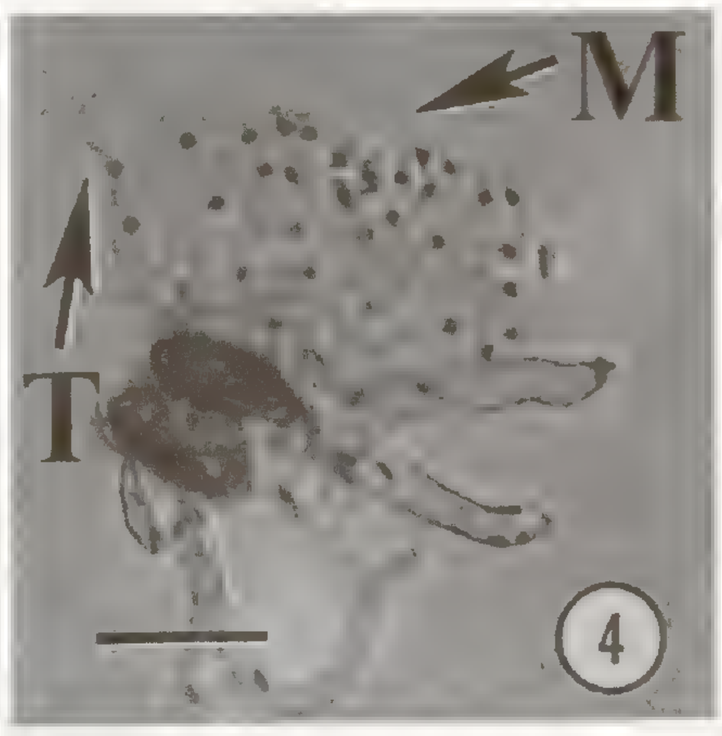
Spore germination began four days after sowing. Some spores germinated as late as nine days and among spores that were in cultures observed daily, none germinated after nine days. Approximately 90% of viable spores had germinated by day seven. A prothallial cell and a single rhizoid were observed emerging from the spore coat at germination. Some gametophytes were two dimensional at six days with two rhizoids formed while others had recently germinated. By day 10, prothalli were either spatulate (Fig. 3) or more fan-shaped (Fig. 4) with 2–5 rhizoids formed at the basal region. Initiation of a terminal organized meristem was evident, with cells of this meristem more thin and elongate when compared to other marginal cells. In some gametophytes, cells at the basal region were large and formed small lobes (Fig. 5). Unicellular marginal trichomes began to develop at this time, located on the outer margins of the prothallus (Fig. 4). From gametophytes observed daily, an organized meristem was initiated 5–9 days after germination in 75% of gametophytes.

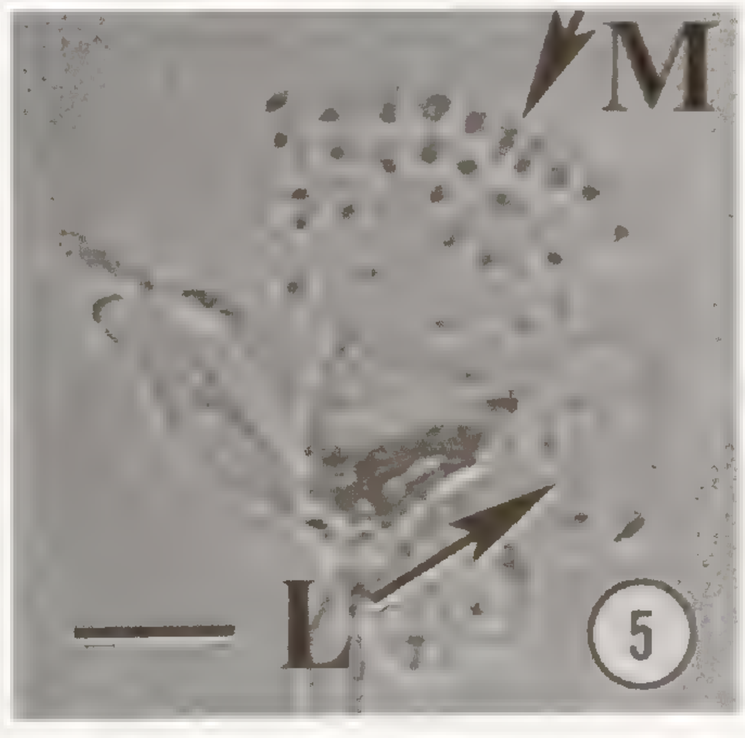
At 12 days, gametophytes varied in size, with some gametophytes considerably larger than others. Larger gametophytes measured 0.2–0.3 mm in width with prominent notched meristems and trichomes along the margin (Fig. 6). Trichomes were slightly swollen at the tip with prominently stained nuclei (Fig. 7). Smaller gametophytes were 0.1–0.2 mm in width. Some lacked an organized meristem, whereas others had an organized meristem, but lacked a notched meristem.

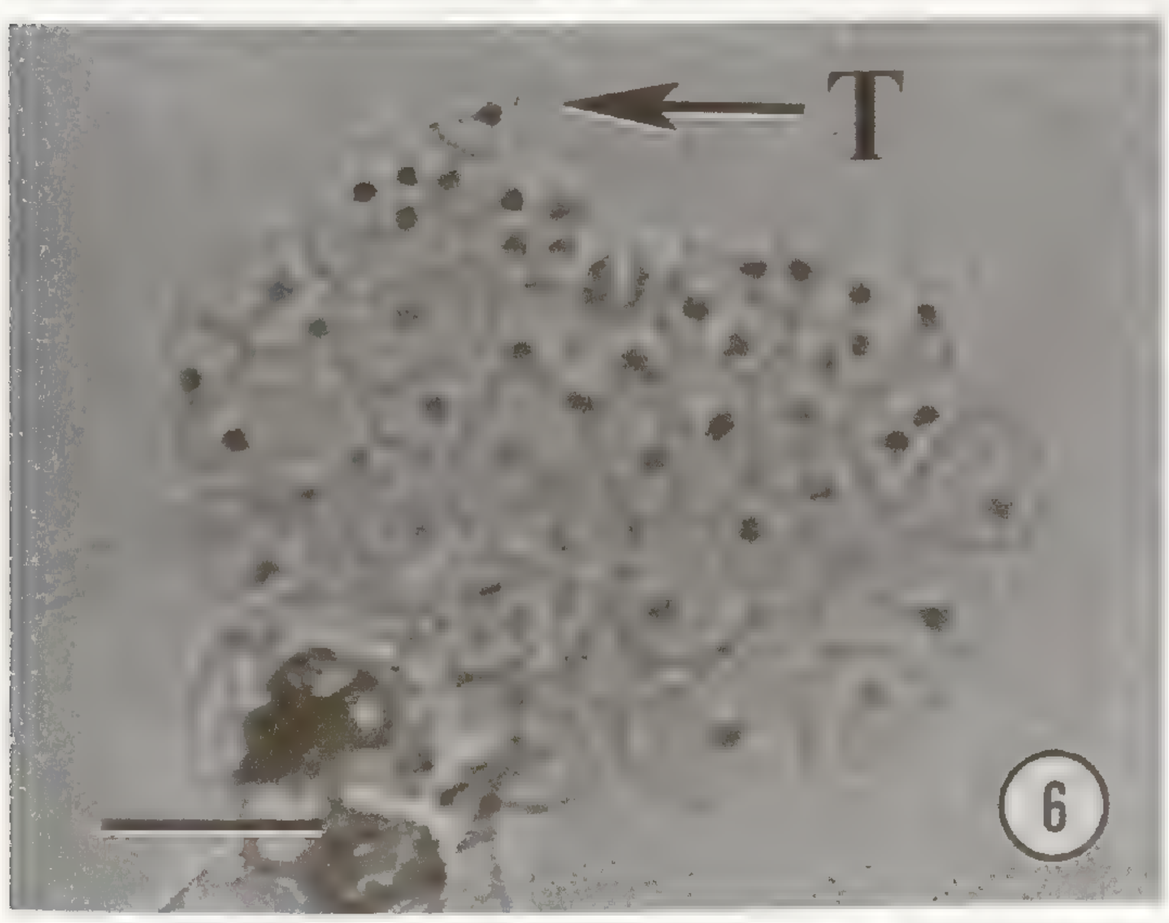
At 19 days a small percentage of gametophytes had begun to form antheridia while the majority of gametophytes remained asexual. Antheridia were formed on the surface of small (0.3–0.6 mm in width) cordate gametophytes near the

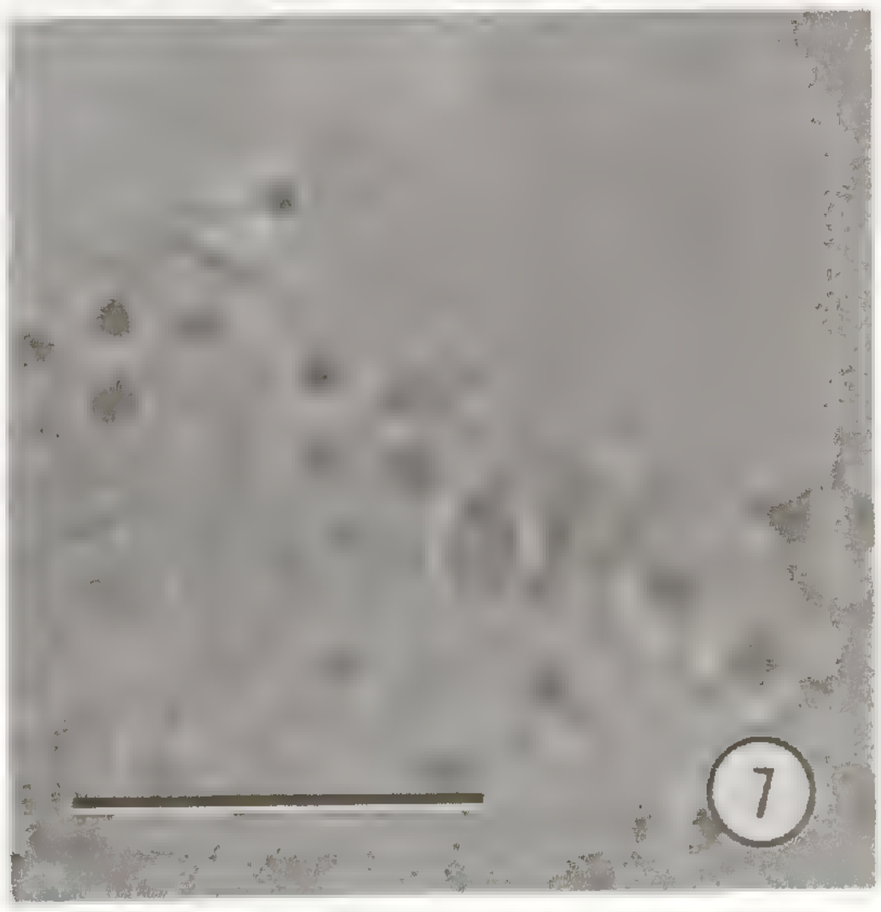












FIGS. 1–7. Spores and young gametophytes of *Thelypteris ovata* var. *lindheimeri*. Fig. 1. SEM of monolete spores with continuous folds. Bar = 25 μm. Fig. 2. SEM of spore with perforations. Bar = 10 μm. Fig. 3. Spatulate gametophyte with organized meristem (M) at 10 days. Bar = 50 μm. Fig. 4. Fan-shaped gametophyte at 10 days with organized meristem (M) and marginal trichome (T). Bar = 50 μm. Fig. 5. Gametophyte at 10 days with organized meristem (M) and basal lobe (L). Bar = 50 μm Fig. 6. Cordate gametophyte at 12 days with marginal trichome (T). Bar = 50 μm. Fig. 7. Enlargement of trichome with dark-staining nucleus from Fig. 6. Bar = 50 μm.

basal region (Fig. 8). Antheridia were spherical and measured 0.04 mm across (Fig. 9).

Young archegonia were distinguished as early as 21 days on gametophytes prepared for light microscopy (Figs. 10, 11). Archegonial necks were observed on living gametophytes at 24 days. Archegonia developed behind the notched meristem and archegonial necks curved away from the notched meristem. At 24 days, archegoniate gametophytes measured 0.9–1.4 mm in width. At this time, numerous multicellular trichomes could be seen developing on the surface of the gametophytes (Fig. 12) as well as along the margin.

At 28 days, gametophytes were antheridiate, archegoniate, or asexual. Antheridia continued to form on the surface of small (0.6–1.0 mm in width) cordate gametophytes (Fig. 13). As many as 20 antheridia formed on some antheridiate gametophytes, and antheridia varied in maturity from those with mature coiled sperm to those in which the spermatogenous tissue was undergoing mitosis (Fig. 14). Archegonia continued to form behind the notched meristem of large (1.3–2.3 mm in width) cordate gametophytes. One to five archegonia formed per gametophyte. Asexual gametophytes varied in size from 0.5 to 1.5 mm in width.

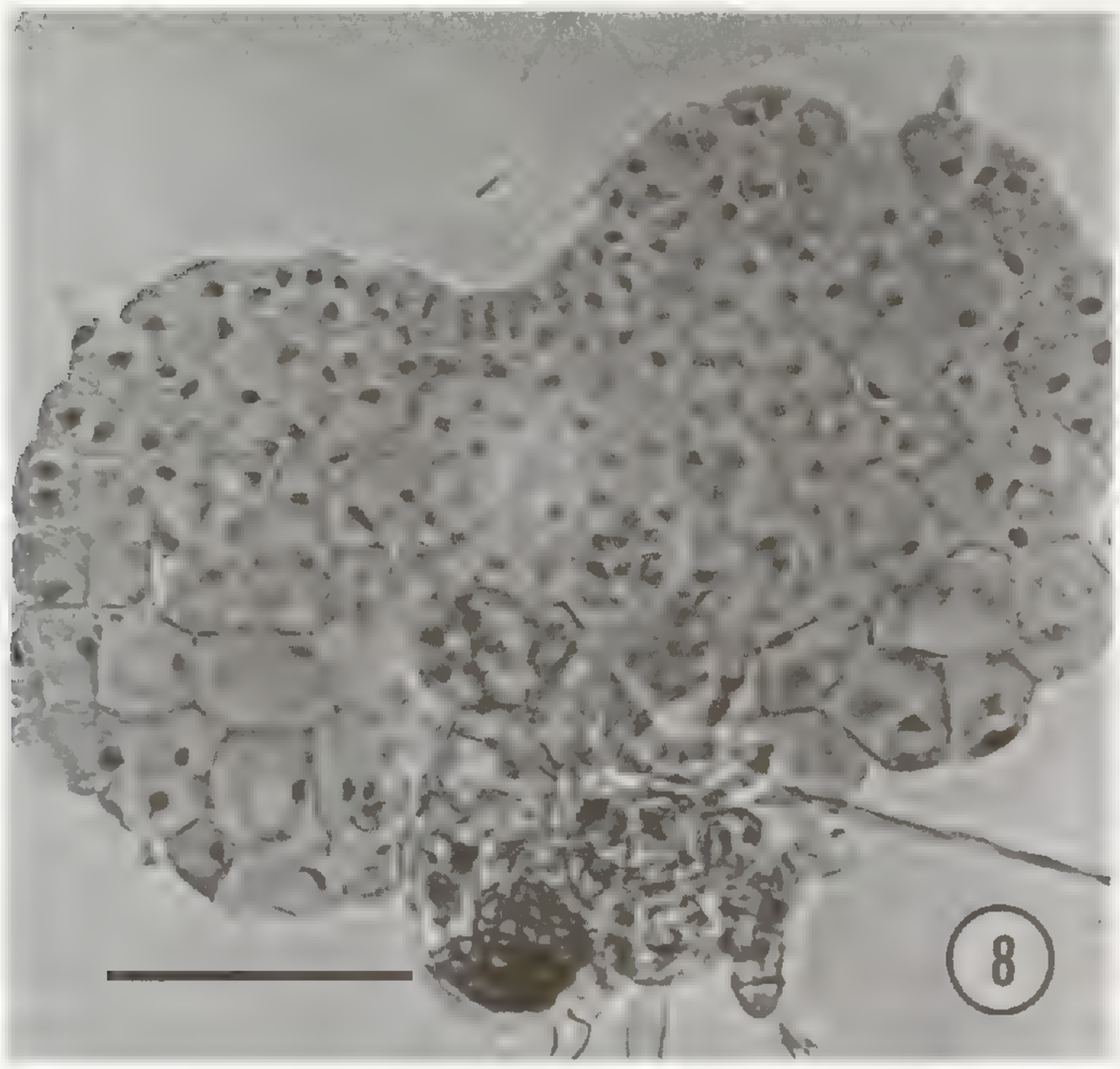
At 48 days, almost all gametophytes were cordate. Approximately 10% of gametophytes were antheridiate while most were archegoniate, and less than 10% remained asexual. No bisexual gametophytes were observed. Throughout this study, archegoniate gametophytes were larger than antheridiate gametophytes and cordate asexual gametophytes were variable in size.

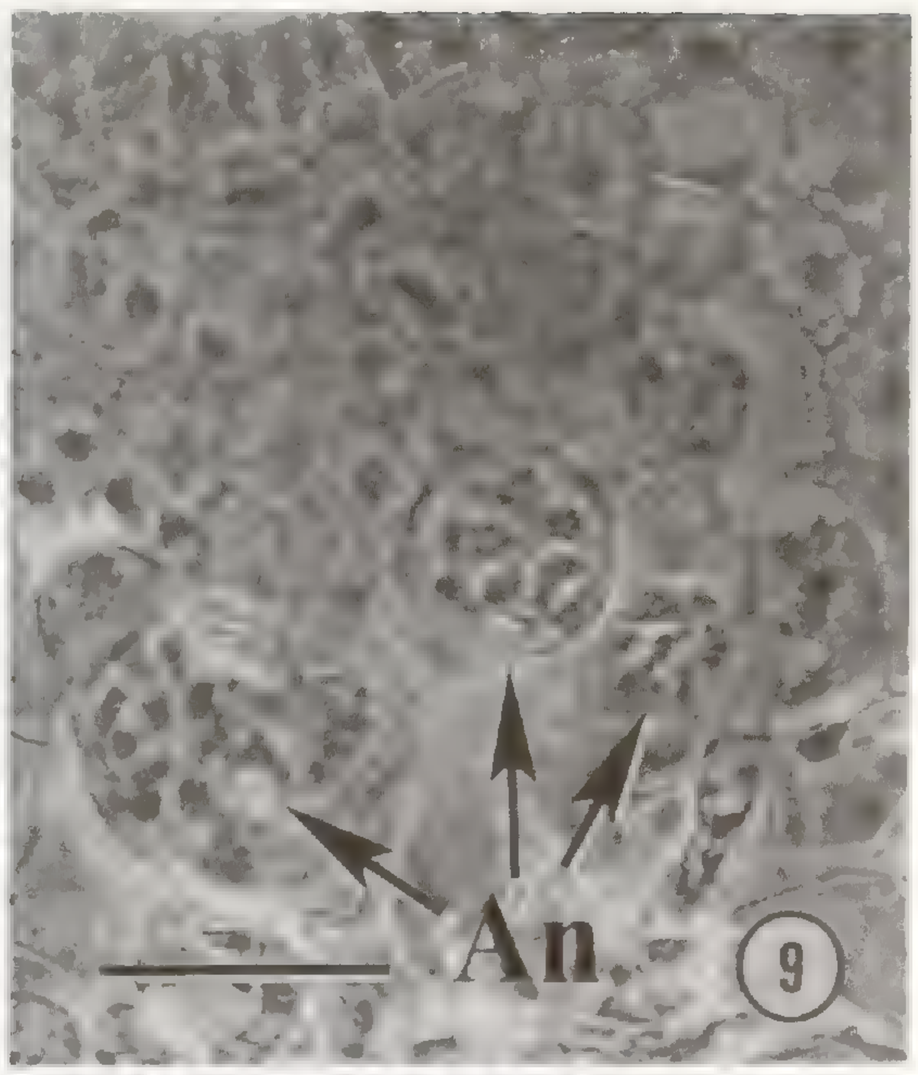
Gametophytes that were not crowded in the Petri plates were flat and cordate with very little ruffling of the wings. Those growing in crowded conditions were often growing in a more upright position. Wings remained one-cell thick with the midrib several cells thick.

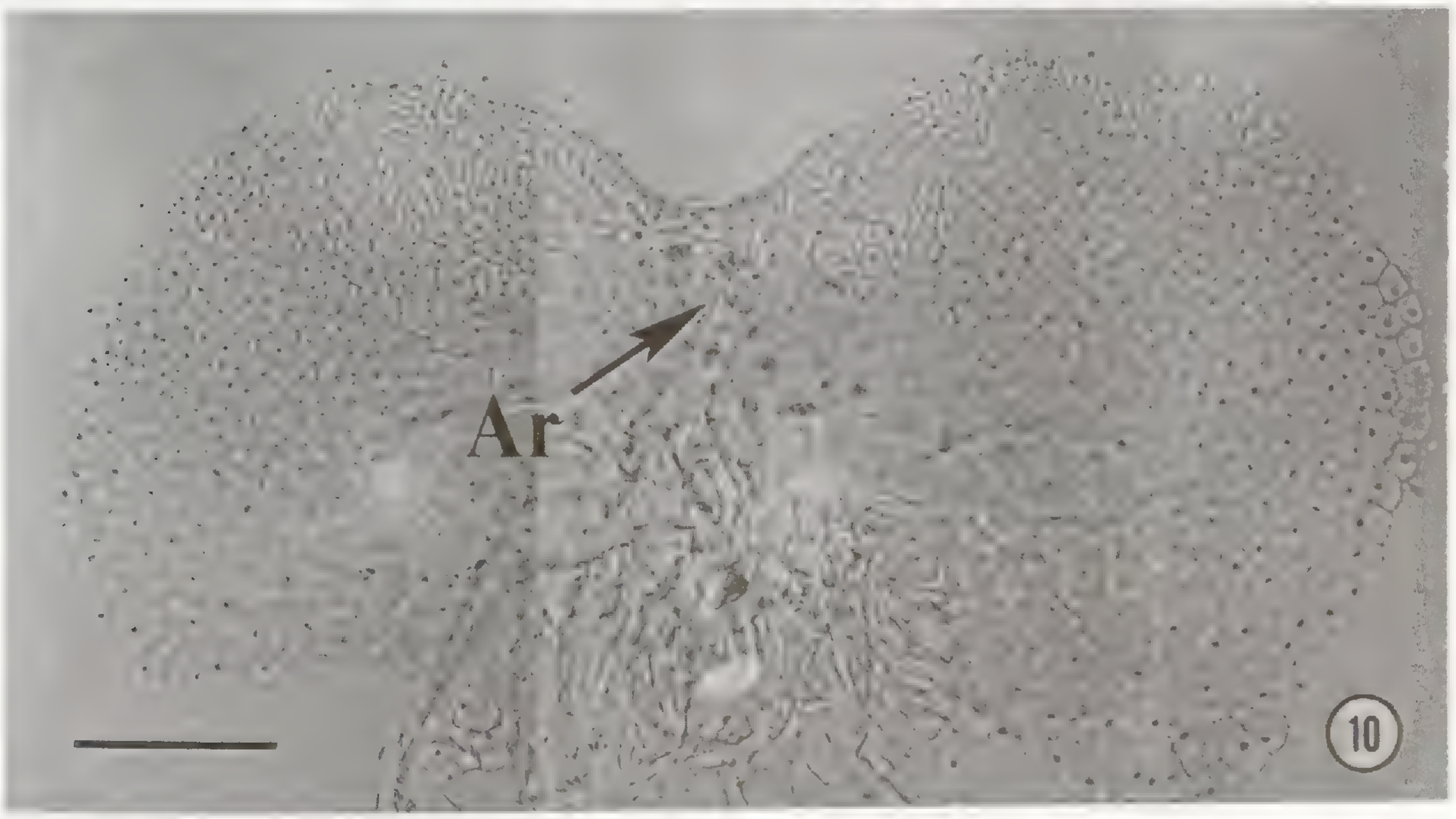
Observations of gametophytes studied daily suggested that time of spore germination or time of initiation of organized meristem had no influence upon whether the gametophyte was antheridiate, archegoniate or asexual at 28 days. Crowding of gametophytes did not appear to influence the number of antheridiate gametophytes.

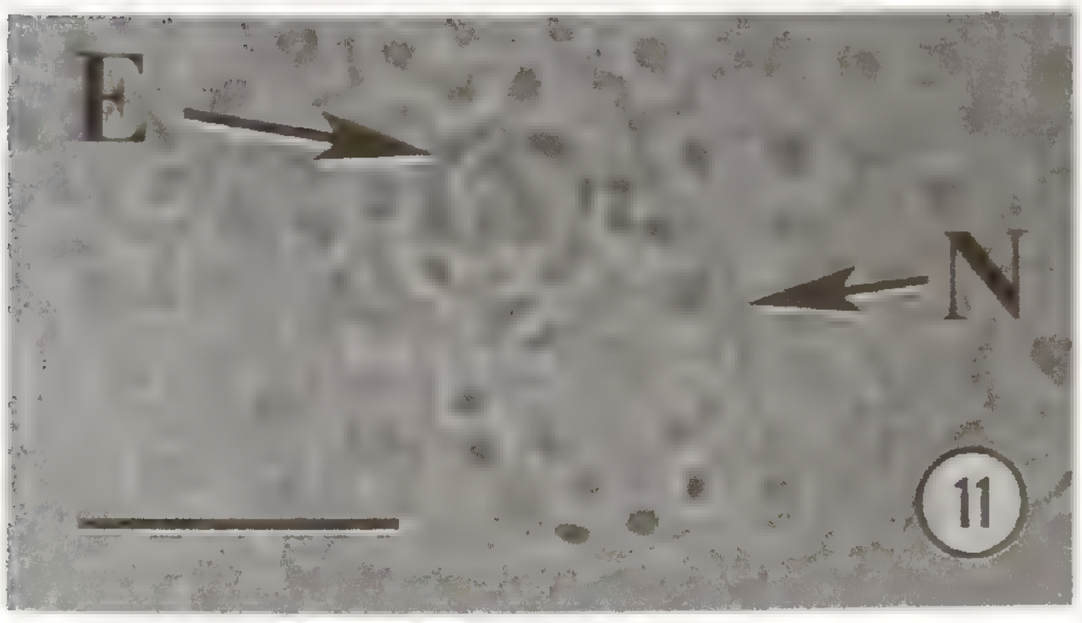
The presence of an antheridiogen system in *T. ovata* had been suggested from previous preliminary studies in our laboratory. Further work involved partial purification of an antheridiogen with TLC. Gametophytes of *T. ovata* and *O. sensibilis* form multiple antheridia prematurely in response to antheridiogen synthesized by *T. ovata* gametophytes (Figs. 15,16). The acidified ethyl acetate extract of culture media after fractionation by TLC showed two bands of biological activity at R_f 0.3–0.4 and 0.8 (Fig. 17). Similar results were obtained with bioassays of *O. sensibilis* gametophytes. Spore germination for both species was greater than 90% in these bioassays.

Spores of *T. ovata* and *O. sensibilis* do not germinate in darkness in response to antheridiogen. In light grown cultures, approximately 90% of spores had germinated by day seven. When these ungerminated spores were subsequently placed in the light and allowed to germinate and grow for 10–14 days, antheridia were initiated. In response to GA_3, no gametophytes of *T. ovata* ger-

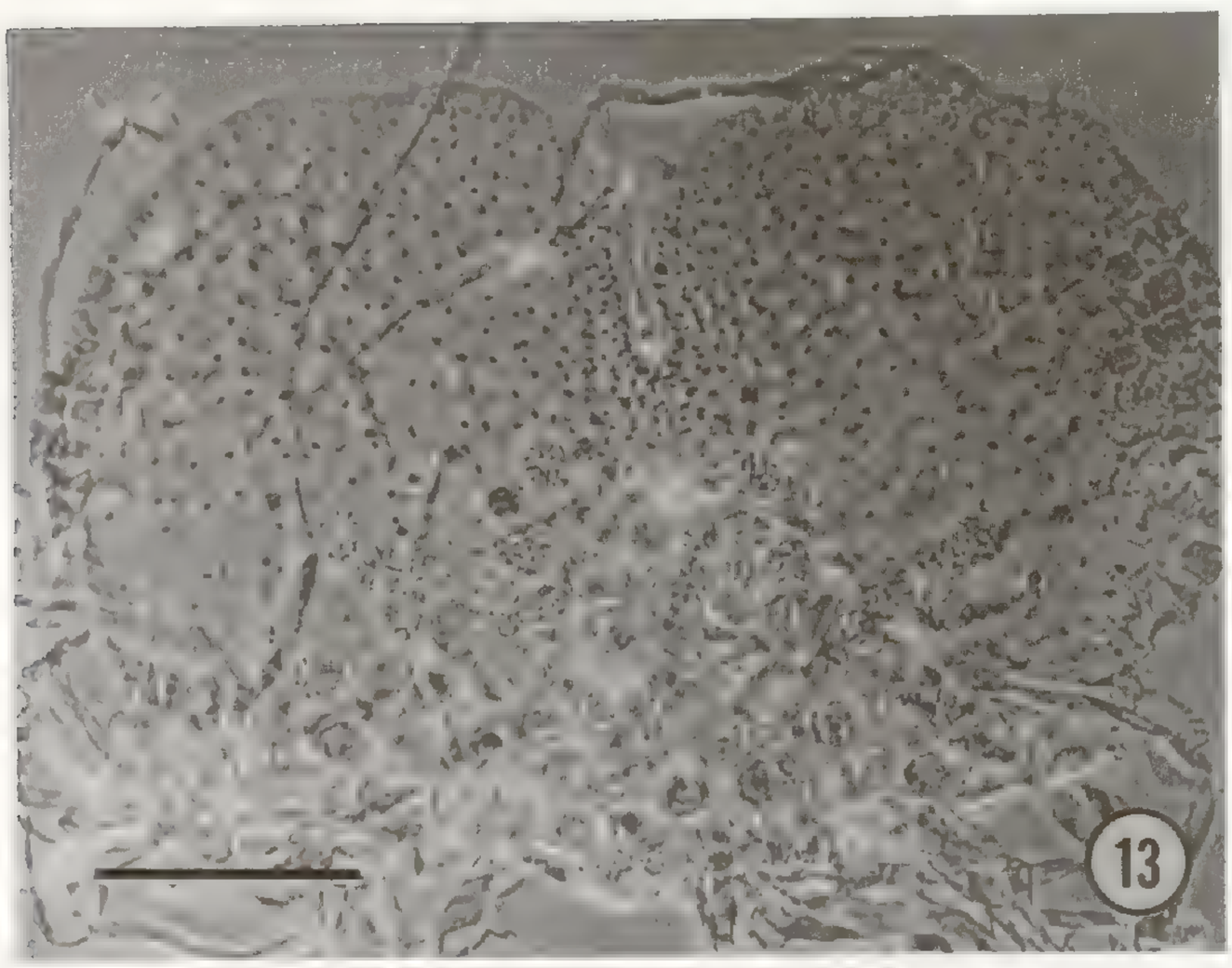


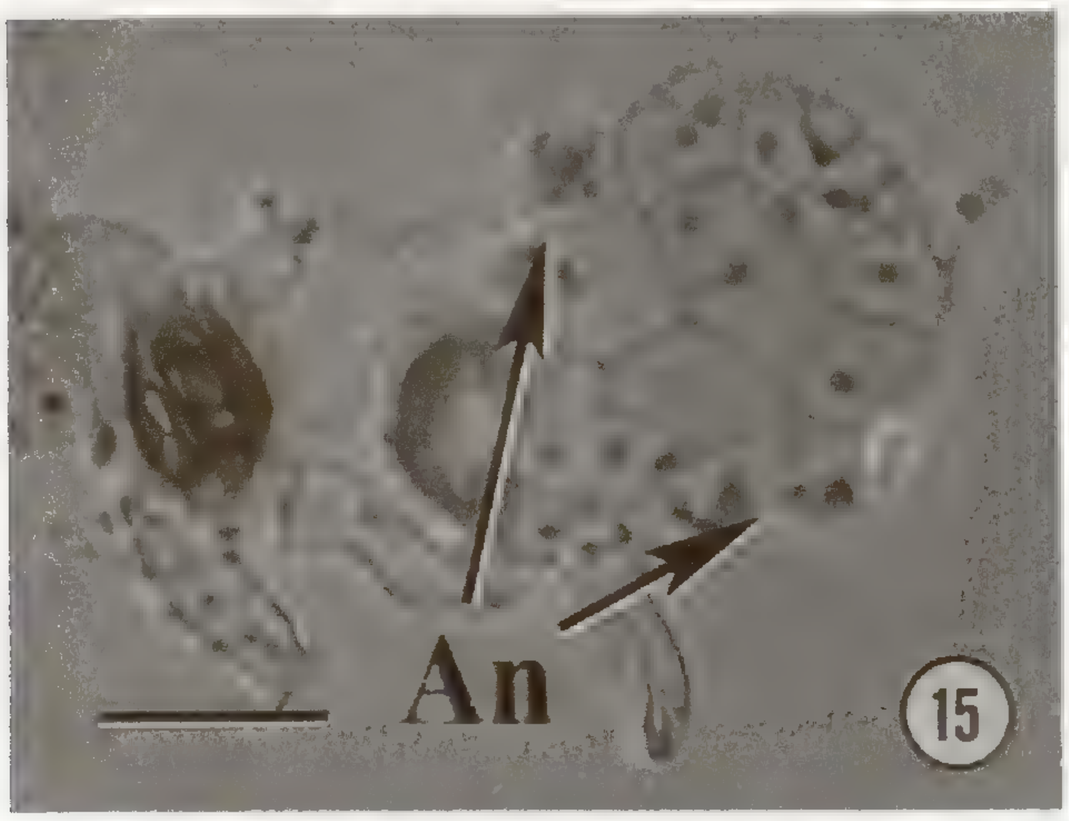


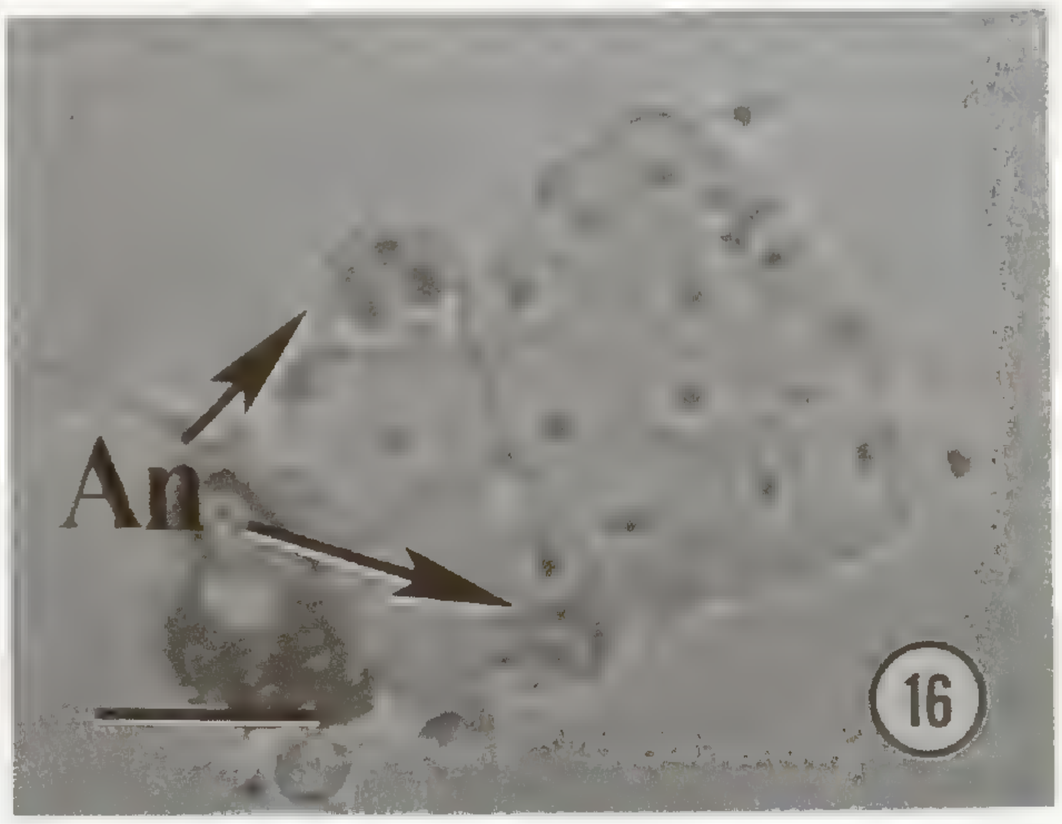




FIGS. 8–12. Gametophytes of *Thelypteris ovata* var. *lindheimeri*. Fig. 8. Cordate gametophyte with antheridia at 20 days. Bar = 100 μm. Fig. 9. Enlargement of antheridia (An) from Fig. 8. Bar = 50 μm. Fig. 10. Cordate gametophyte with archegonium (Ar) at 24 days. Bar = 200 μm. Fig. 11. Enlargement of archegonium from Fig. 10 with archegonial neck (N) and egg (E). Bar = 50 μm. Fig. 12. Surface trichomes (T). Bar. = 50 μm.







FIGS. 13–16. Gametophytes of *Thelypteris ovata* var. *lindheimeri*. Fig. 13. Antheridiate gametophyte at 28 days. Bar = 200 μm. Fig. 14. Enlargement of antheridia from Fig. 13. Bar = 50 μm. Figs. 15, 16. Fourteen day old gametophytes which have formed antheridia (An) prematurely in response to antheridiogen partially purified by TLC. Bar = 50 μm.

minated in darkness by day seven or formed antheridia in the light at any GA_3 concentration used in this study.

DISCUSSION

Spores of *T. ovata* var. *lindheimeri* are monolete with distinct continuous folds. This spore morphology is similar to *T. ovata* var. *ovata, T. flaccida* (Blume) Ching, and *T. reptans* (J. F. Gmel.) C. V. Morton (Tryon and Lugardon, 1991). This general morphology also has been described for *T. cordata* (Fée) Proctor and *T. brunnea* (C. Chr.) Ching (Wood, 1973). Some *Thelypteris* species have spores with short spines on the surface (Wood, 1973; Tryon and Lugardon, 1991). These spines are not present on *T. ovata* var. *lindheimeri* spores.

Spore germination occurred 4–9 days after spore sowing. In other *Thelypteris* species, this also has been observed (Kachroo, 1963; Smith, 1971). The pattern of cell divisions at germination has been studied by Huckaby and Rag-

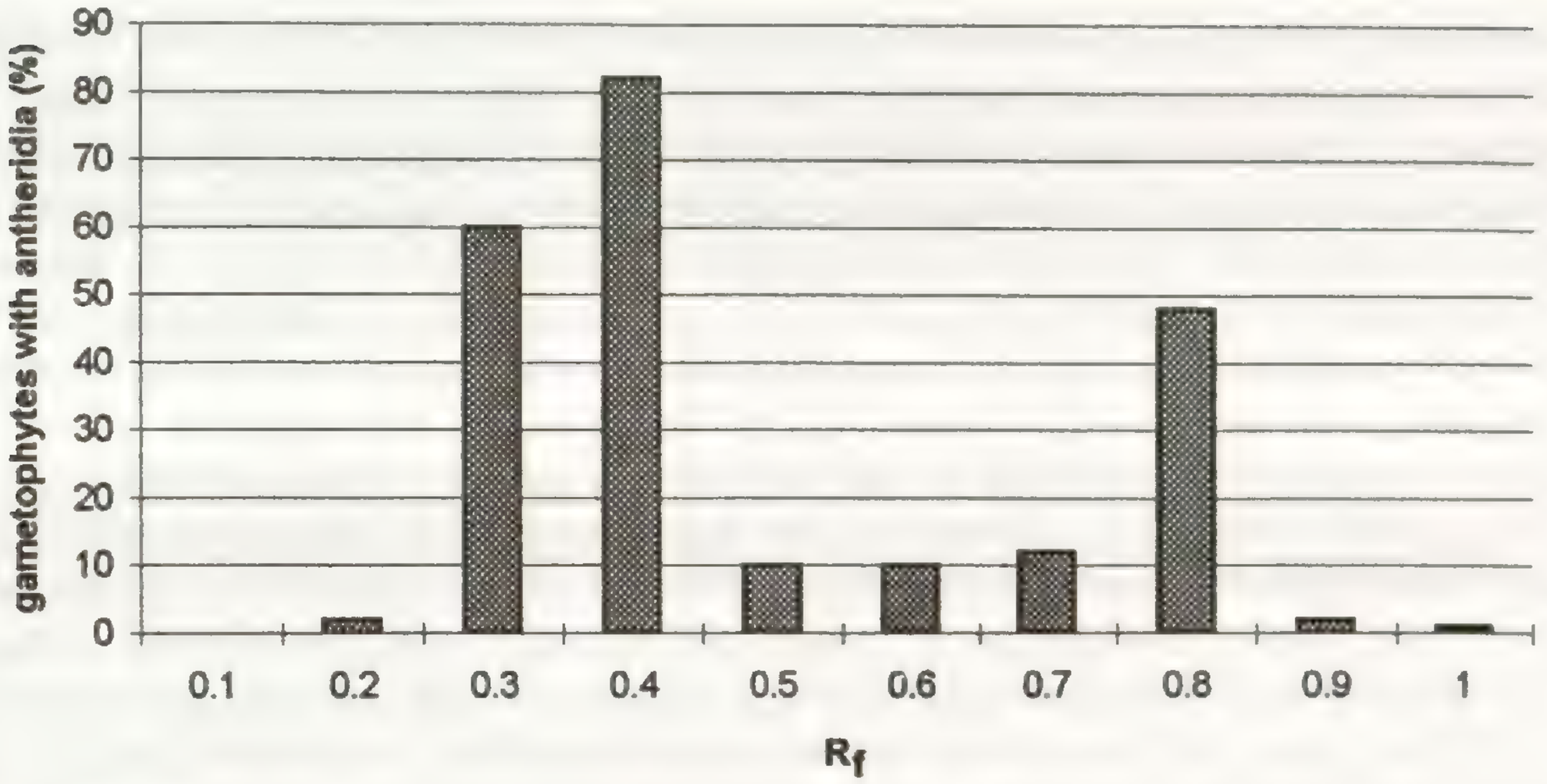


FIG. 17. Percentage of *T. ovata* gametophytes with antheridia in response to gametophyte culture media extract of *T. ovata* after fractionation by TLC (ethyl acetate:chloroform:acetic acid, 75:25:5).

havan (1981) for several *Thelypteris* species using both sectioned material for light microscopy and intact material for SEM. In their work, it was found that the spore coat cracked open along the monolete mark and the first asymmetric division of the spore cell resulted in a rhizoid cell and basal cell. The basal cell divided again to form a protonemal cell. In the present study, the cell division pattern at germination could not be determined because sectioned material was not studied.

Gametophytes of *T. ovata* did not form a noticeable filament and were two-dimensional almost immediately. This differs from most other *Thelypteris* species that have been studied. In *Cyclosorus molliusculus* (Kuhn) Ching, spores germinated seven days after sowing and gametophytes were initially a three-celled filament (Kachroo, 1963). In several *Thelypteris* species, a three to six-celled filament formed (Nayar and Chandra, 1963; Atkinson, 1971, 1975; Atkinson and Stokey, 1973).

At 12 days, gametophytes of *T. ovata* have well-developed organized meristems and several unicellular marginal trichomes (Fig. 6). Gametophyte development in *T. ovata* follows the *Drynaria* type described by Nayar and Kaur (1971). Unicellular marginal and surface trichomes have been described in other *Thelypteris* species, although time of initiation of trichomes varied between species (Kachroo, 1963; Nayar and Chandra, 1963, 1965; Atkinson, 1971, 1975; Smith, 1971; Atkinson and Stokey, 1973). In *T. erubescens* (Wall. ex Hook.) Ching, some gametophytes have marginal trichomes at one month, but others lack trichomes (Atkinson, 1971). No glandular secretions were observed. Acicular trichomes have been reported in some *Thelypteris* species (Atkinson and Stokey, 1964).

In *T. ovata,* antheridia and archegonia are on separate male and female gametophytes when gametophytes are grown in multispore cultures. Archegoniate gametophytes are considerable larger than antheridiate gametophytes.

Antheridia and archegonia were observed on separate gametophytes throughout this study. Antheridia began to form at 19 days and archegonia began to form at 21 days. In *T. erubescens,* archegonia were observed on gametophytes after two months and antheridia were observed on separate, smaller gametophytes (Atkinson, 1971). In the *Thelypteris* sp. studied by Näf (1956), antheridia were not formed at any stage of development. In this study, approximately 80% of gametophytes were archegoniate, 10% antheridiate, and 10% asexual at 48 days.

Mature gametophytes of *T. ovata* are similar to other mature *Thelypteris* gametophytes described previously, but few details of gametophyte development in the first 30 days are available for these species. Information concerning the time of spore germination, initiation of organized meristem, and initiation of antheridia and archegonia is limiting. Trichome and overall gametophyte morphology can be useful when attempting to identify fern gametophytes in nature (personal observation), and Atkinson and Stokey (1964) presented evidence that fern gametophytes could be used taxonomically. Differences between gametophytes of various species can only be determined through detailed studies of growth, development, and morphology.

The presence of antheridiate and archegoniate gametophytes at the end of 48 days suggested that an antheridiogen system may be present in this species. Preliminary studies in our laboratory verified the presence of an antheridiogen system and partial purification of the antheridiogen was undertaken. The results of bioassays with *T. ovata* and *O. sensibilis* indicate the presence of more than one biologically active compound. This is not unusual as two or more antheridiogens have been detected and/or chemically characterized in *Anemia phyllitidis* (L.) Sw. (Oyama, et al., 1996), *A. mexicana* Klotzsch (Nester et al., 1987), *Lygodium japonicum* (Murr.) Sw. (Yamane et al., 1979, 1988), *L. circinnatum* (Burm. f.) Sw., and *L. flexuosum* (L.) Sw. (Yamauchi, et al., 1996). The biologically active compounds of *T. ovata* will be purified further by ion-exchange chromatography and high performance liquid chromatography for eventual chemical characterization.

Spores of *T. ovata* and *O. sensibilis* are not induced to germinate in darkness in response to antheridiogen of *T. ovata.* These results are as expected when compared to results of antheridiogen studies in most other non-schizaeaceous ferns. However, Chiou and Farrar (1997) recently demonstrated that antheridiogens in the Polypodiaceae can induce spore germination in darkness after 30 days. Since greater than 90% of *T. ovata* spores germinate in the light by day seven, bioassays were observed at day seven.

Spores of *T. ovata* are not induced to germinate in darkness in response to GA_3. These results also are expected when compared to results of GA_3 activity in most other non-schizaeaceous ferns.

Antheridiogen systems have been described in other fern species (review by Näf et al., 1975; Raghavan, 1989). *Thelypteris hexagonoptera* and *Thelypteris* sp. have been shown to be sensitive to the antheridiogen of *P. aquilinum,* and the presence of an antheridiogen in *T. hexagonoptera* was demonstrated using *O. sensibilis* as the bioassay organism (Näf, 1956). Although the *Thelypteris*

sp. studied by Näf (1956) was sensitive to the antheridiogen of *P. aquilinum,* the presence of an antheridiogen was not demonstrated in that species. This study is the first to report an antheridiogen in *T. ovata* and the second detected in the large family Thelypteridaceae, of which there are approximately 1000 species. Further work will require biological and chemical characterization of these compounds. In the search for new ferns with antheridiogens, studies of gametophyte growth, development and reproductive morphology will be useful for providing baseling information for individual species. Percentages of antheridiate and archegoniate gametophytes at maturity may differ between ferns. For example, in *A. mexicana,* approximately 80% of cordate gametophytes are antheridiate, 10% are archegoniate and 10% are asexual at 60 days (Nester and Schedlbauer, 1981). This information is essential for discussions of gametophyte reproductive strategies and can only be obtained by detailed studies of growth and development.

ACKNOWLEDGMENTS

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Photosynthetic Capacity and Leaf Reorientation in Two Wintergreen Ferns, *Polystichum acrostichoides* and *Dryopteris intermedia*

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ABSTRACT.—Some ferns growing in areas with severe winters maintain green leaves through the winter; however, very little is known about the physiology of their remarkably hardy leaves, and the function of the wintergreen trait has not been completely established. Both *Polystichum acrostichoides* and *Dryopteris intermedia* retain high concentrations of chlorophyll (2.4 mg/g fresh weight) and a high quantum efficiency (0.6–0.8) in photosystem II. Even after a winter, the *P. acrostichoides* leaves show a significant photosynthetic rate (ca. 2 μmoles $CO_2/m^2/sec$). During fall, a 1 to 2-cm zone in the lower part of the stipe senesces and softens forming a hinge that allows the leaves to sink to the ground. This prostration does not cut off their xylem flow, and it apparently minimizes their exposure to cold and desiccation.

A number of ferns growing in temperate climates with a cold winter are wintergreen (Wherry, 1961), and this appears to represent an advantageous adaptation. Wintergreen refers to plants that maintain green leaves through a single winter, after which they die as they are replaced by a new set of leaves (Wagner, 1996). In Michigan, 24 (31%) species of ferns have fully wintergreen leaves, 4 (5%) are partially wintergreen, and 49 (64%) have leaves that die before winter each year (W. H. Wagner, unpublished data).

One obvious possible value of the wintergreen trait is extension of the photosynthetic period, but this trait may also enable the plants to retain limiting nutrients and to redistribute them from the old leaves to the new leaves more efficiently (Moore, 1984; Van Buskirk and Edwards, 1995). The importance of wintergreen leaves as photosynthetic organs in climates with severe winters has been questioned (Moore, 1984). Nonetheless, the value of the wintergreen leaves is clearly demonstrated in *Dryopteris intermedia* (Muhl.) A. Gray where removal of the old leaves in the early spring (April 1) before the new growing season greatly suppresses (60% reduction) the new growth (Van Buskirk and Edwards, 1995). Exactly what these leaves contribute after April 1 and their role in general remains to be established.

Not only is the wintergreen phenomenon an interesting aspect of natural history that needs further study, but the wintergreen leaves appear to be resistant to some extreme environmental stresses, and understanding the nature of this resistance may be of practical value in offering ways to engineer crop plants to increase stress resistance.

Both the Christmas fern, *Polystichum acrostichoides* (Michx.) Schott, and the "evergreen" or glandular wood fern, *Dryopteris intermedia* (Muhl. ex Willd.) A Gray, remain green and fresh in appearance through the winter in northeastern U.S.A.; however, only very limited data are available on their

photosynthetic activity during this time (Minoletti and Boerner, 1993). This paper examines in a preliminary way their winter photosynthesis and some related phenomena. We also address some of the remarkable mechanical adaptations that these ferns appear to use to protect their leaves and their photosynthetic machinery from damage by the cold and desiccation that occur during winter.

Methods

Whole *Polystichum acrostichoides* plants or leaves alone were collected from one isolated clump of plants in an oak forest near Ann Arbor. *Dryopteris intermedia* leaves were collected from a rich, mixed woodlot near Ann Arbor. The plants or whole detached leaves were stored in darkness at 5°C in a plastic bag with a moist (not wet) paper towel until they were analyzed within 48 hr.

Chlorophyll was extracted by placing the leaflets in dimethylformamide and quantified spectrophotometrically as described by Canfield et al. (1995). Photosynthesis was measured as net CO_2 uptake at 20°C, 20% relative humidity, and 600 mol/m^2/sec red light (ca. 670 nm) with an infrared gas analyzer, a LI-COR LI 6400 portable photosynthesis meter (LI-COR, Inc., Lincoln, NB) as described by Guiamét et al. (1990). The quantum efficiency (Fv/Fm) of photosystem II was measured at 25°C with an OS-500 Modulated Fluorimeter (PP Systems, Haverhill, MA 01830).

To study xylem flux and the integrity of the xylem system, leaves were cut at their base, below the trophopod (the swollen basal region in the leaf axis [cf. Wagner and Johnson, 1983 and Fig. 1B]), and they were placed in a solution of the vital dye trypan blue (0.4% w/v in water [Artis et al., 1985]). The leaves were supported vertically in an indoor laboratory at 25°C, 25% relative humidity and 11 mol/m^2/sec from cool white fluorescent lights.

Results

During the fall (November in the Ann Arbor, Michigan area), the leaves of *P. acrostichoides* and *D. intermedia* decline from an upright to a prostrate posture (Fig. 1A). This change is caused by softening of a 1–2 cm zone in the lower stipe above the trophopod (Fig. 1B), and, thereafter the stipe no longer supports the weight of the leaf, forming a hinge and bending at the softened zone. In contrast to the rest of the stipe, this softened zone can be compressed easily by squeezing it between the fingers. The surface of this hinge zone is brown-colored, whereas the neighboring stipe surface is green. Except for the vascular sheath, the cells in the hinge are dead, and they are friable, i.e., loosely connected.

If dye (trypan blue) was supplied through the base of the stipe cut off below this soft zone, the dye was pulled up into the leaf blades by transpiration. When the stipe was viewed in cross section, it could be seen that the dye was restricted to the xylem, i.e., it did not leak out into the surrounding tissues

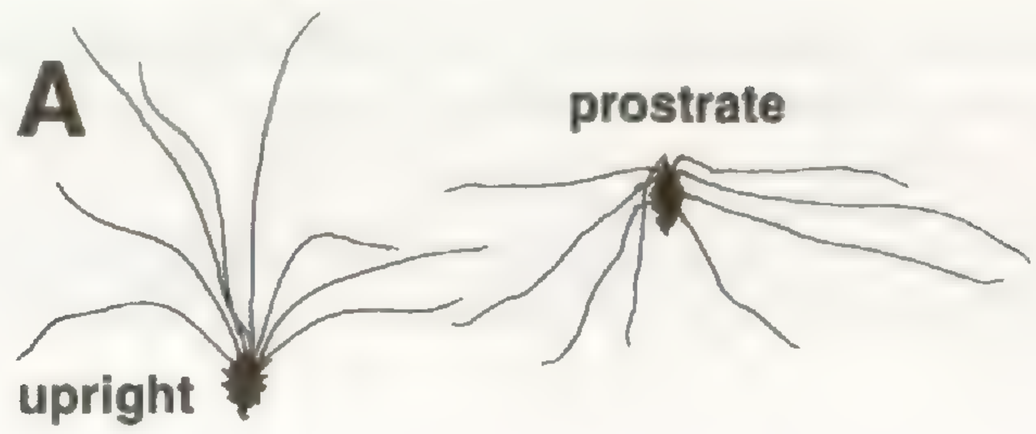

FIG. 1. *Polystichum acrostichoides* leaves. A) Prostration of the leaves; B) Location of the hinge (soft zone) that causes the prostration; C) Cross sections of the stipe showing the vascular bundles (circles) and dye location marked by Xs inside the circles. In order to simplify and clarify these diagrams (A), only the major axes are shown; the leaflets have been omitted.

(Fig. 1C). Unlike the other stipe tissues, most of the tissue in this elbow was dead and quite friable. As would be expected from the permeability results, the sheath around the vascular tissue appeared to be alive.

The chlorophyll concentrations in these leaves in late winter (Feb. 12, 1996) following some periods of relatively low temperature (ca. −10°C) without snow cover remained high, on par with soybean, *Glycine max* (L.) Merr. (Table 1) during its peak photosynthesis. Soybean was selected for comparison here, because of its high photosynthetic activity. The chlorophyll a/b ratios were 2.35 and 2.03 for *P. acrostichoides* and *D. intermedia* respectively compared with 2.95 for *G. max*. At this time, *P. acrostichoides* and *D. intermedia* showed PSII quantum yield of 0.6–0.8, which is similar to 0.8 for *G. max* (Table 2).

Later, in early spring (Apr. 5), most of the leaves of *P. acrostichoides* were still fresh and green in appearance, and they carried on net CO_2 fixation at a rate of about 2 mol/m²/sec at 20°C compared with about 20 mol/m²/sec for *G. max* at 25°C (Table 3). Interestingly, discolored (darkened) *P. acrostichoides* leaves still had substantial amounts of chlorophyll (50–75%), and the chlorophyll a/b ratios were similar to the normal, green leaves, yet they were essentially inactive in terms of CO_2 uptake or release (Table 3).

During late winter through mid spring, i.e., Feb. 12 through Apr. 27, the leaves of *P. acrostichoides* not only failed to increase their dry weight (biomass), but they actually lost some dry matter (Table 4).

TABLE 1. Chlorophyll concentrations in the leaves of two stay-green ferns in late winter (Feb. 12, 1996) compared to soybean. [a]Data from Guiamét et al. (1991).

	mg Chl/g fresh weight	g Chl/cm^2	Chl a/b
Polystichum acrostichoides	2.4 ± 0.2	44.3 ± 2.7	2.35 ± 0.04
Dryopteris intermedia	2.3 ± 0.05	N.D.	2.03 ± 0.06
Glycine max	N.D.	50[a]	2.95[a]

DISCUSSION

Those wintergreen ferns that grow in climates having a cold winter are particularly impressive among wintergreen species. They seem to be delicate, yet they are able to endure very harsh winter conditions, i.e., cold and desiccation. One factor in their durability is their ability to reposition their leaves to a less exposed posture. During fall, *P. acrostichoides* and *D. intermedia* leaves change their posture from upright to prostrate (Fig. 1A). Although this change in the orientation of certain fern leaves has been noted incidentally (Parsons, 1899), it seems not to have been studied in any detail. This reorientation undoubtedly reduces their exposure to cold, drying winds (Givnish, 1982); however, these prostrate leaves could still carry on photosynthesis, at least while uncovered on warm, sunny days.

To produce this reorientation, a 1–2 cm zone in the stipe, near the ground but above the trophopod (Fig. 1B), softens and is unable to bear the weight of the leaf. This soft zone becomes a hinge causing the leaf to lie flat on the ground. Even though most of the supporting tissue in this hinge has died and collapsed, dye movement indicates that the vascular strands themselves are not blocked and remain functional. Moreover, because the dye used, trypan blue, is an indicator of cell integrity (Artis et al., 1985), we were also able to determine that the xylem symplastic domain retains its integrity, that is the solutes do not leak out. The hinge zone seems to form through a localized senescence or apoptosis.

Two quite different explanations have been proposed for the function/benefit of the wintergreen character (Moore, 1984; Van Buskirk and Edwards, 1995). First, the wintergreen leaves may extend the period of photosynthesis for these plants. It may be a considerable advantage for these understory species to carry on photosynthesis when the leaves of the forest canopy are absent; however, the plants may have to pay a "cost" for developing cold resistant leaves and for maintaining these leaves during the winter. A second idea relates to storage and transfer mechanisms. Basically, it may cost less for these organisms to retain limiting nutrients in their old leaves and redistribute them directly to new leaves after the winter (one transfer) than to transfer them first to specialized storage tissues in the rhizome and/or trophopod and then to new leaves in the spring (2 transfers). Each transfer step presumably has a cost. Removal of the foliage from *D. intermedia* during the winter shows that the old foliage makes an important contribution to the growth of the new leaves

TABLE 2. Light-harvesting capacity (quantum yield of PSII) in the leaves of two stay-green ferns in late winter (Feb. 12, 1996) compared to soybean. [a]Unpublished data from Guiamét et al. (1995).

	F_v/F_m
Polystichum acrostichoides	0.55–0.71
Dryopteris intermedia	0.59–0.76
Glycine max	0.78–0.81[a]

(Van Buskirk and Edwards, 1995), but this could involve either or both of the two mechanisms cited.

Determination of the chlorophyll concentrations shows that the wintergreen leaves of *P. acrostichoides* and *D. intermedia* maintain a high concentration of chlorophyll even into the spring. The chlorophyll a/b ratio in fern leaves is similar to but slightly lower than the ratio for *G. max* leaves at their photosynthetic peak. The lower chlorophyll a/b in ferns indicates that they have a slightly larger light-harvesting or antenna system which would be advantageous in shady conditions (Guiamét et al., 1991; Scheer, 1991; Sundqvist and Ryberg, 1993). The high quantum efficiency of photosystem II in these leaves indicates that these chlorophylls are not only retained, but they function efficiently (Scheer, 1991; Sundqvist and Ryberg, 1993; Bolhàr-Nordenkampf and Öquist, 1993). Clearly, the cold caused no significant damage to PSII, which is a particularly sensitive indicator of cold stress.

We and Minoletti and Boerner (1993) find that the leaves of *P. acrostichoides* also retain their capacity for CO_2 fixation through the winter. The CO_2 fixation rates we observed were only 10% of those measured for soybean leaves (Guiamét et al., 1990), and they were also less than other understory herbs (Minoletti and Boerner, 1993); however, our rates were similar to those reported by Minoletti and Boerner (1993) for *P. acrostichoides*. The fact that PSII and net CO_2 fixation are active during late winter and early spring certainly supports the idea that wintergreen leaves could make an important photosynthetic contribution to the plant and to new growth in the spring. Nonetheless, the fact that these leaves fail to accumulate dry matter does raise some questions about what this photosynthesis contributes. If the production of new carbohydrate by photosynthesis exceeds the consumption of carbohydrate for self maintenance, then photosynthate must accumulate outside the leaf blades, e.g., in the rhizome and/or the trophopod.

These data do not exclude the second function of wintergreen leaves, i.e., temporary storage of mineral nutrients. Usually, but not always, leaf senes-

TABLE 3. Photosynthetic rates and chlorophyll contents in the leaves of the stay-green fern (*Polystichum acrostichoides*) in early spring (April 5, 1996).

	moles/m²/sec	g Chl/cm²	Chl a/b
Fresh green leaves	1.81–2.24	39–59	2.20–2.35
Darkened (discolored)	0.06	29	2.21

TABLE 4. Changes in the dry weight (biomass) of the leaves of the stay-green fern (*Polystichum acrostichoides*) during the late winter through early spring.

Date	Dry weight (mg)/leaflet	Dry weight (mg)/cm^2
Feb. 12, 1996	35 ± 3	7.1 ± 0.1
Apr. 27, 1996	18 ± 2	5.1 ± 0.3

cence is accompanied by the removal/release of stored nutrients such as nitrogen and phosphorous (P) from the senescing leaves (Noodén, 1988a,b). A seasonal profile of phosphorous content as g P/kg dry matter in leaves has been reported for *P. acrostichoides* (Minoletti and Boerner, 1993), but their data do not permit clear conclusions about redistribution from the leaves. A measure of P/leaf or P/unit of leaf area would be better. Because the dry weight content of the leaves decreases, at least under our conditions, the small decreases reported for g P/kg dry matter could represent some redistribution of P within the plant; however, this decrease is small and is not reciprocally related to the growth of the new leaves. Thus, the mineral nutrient distribution in these plants needs further analysis, and the trophopods and rhizomes will have to be included. In addition, why these leaves survive the winter only to die after the weather becomes favorable is also an open question.

ACKNOWLEDGMENTS

We thank Dr. James A. Teeri of the University of Michigan Biological station for the loan of the LI-COR LI 6400 IRG meter, and Juan Guiamét and David Rothstein for their help in making the measurements of CO_2 fixation. We also thank Dr. J. Flore of Michigan State University for the loan of the OS-500 Modulated Fluorimeter.

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American Fern Journal 87(4):150–152 (1997)

Shorter Note

***Botrychium virginianum* in California.**—On June 23, 1996, I found a population of *Botrychium virginianum* (L.) Sw. (rattlesnake fern) in the Salmon Mountains of southwestern Siskiyou County, California, about 15 km south of the town of Etna (Etna Quadrangle, T40N R9W, section 7 or 8). The plants were in a wooded, boggy area at about 1300 m elevation, on the west side of Forest Service Road 41N14 (High CCC Road), less than three km northwest of its junction with Tiger Creek Road. A small stream crosses the road at the north side of the boggy area. At least five plants of *B. virginianum* were observed, including two alongside the road, although only a small part of the wettest area was searched. One specimen was collected (*Game 96/043,* UC). In addition to *B. virginianum,* several plants of *Botrychium multifidum* (S. Gmel.) Rupr. were present at the same site (*Game 96/044,* UC). Associated angiosperm species included a conspicuous population of *Lilium pardalinum* and the orchids *Platanthera leucostachys* and *Listera convallarioides.*

Botrychium virginianum is widely distributed in the northern hemisphere, but has not been documented previously in California. It is not treated in any of the standard floras of the state and the nearest documented populations are in Oregon. Populations were mapped in northern Oregon by David H. Wagner (Guide to the species of *Botrychium* in Oregon, unpubl. manuscript, University Herbarium, Dept. of Biology, University of Oregon, Eugene). Additional sites for the species are in the central Oregon Cascades (Lane County) and in Douglas County in southern Oregon (*Rare, threatened and endangered plants and animals of Oregon,* Oregon Natural Heritage Program, Portland, 1991). However, Warren H. Wagner recalls (pers. comm.) seeing *B. virginianum* in northern California several decades ago, but did not collect a specimen. His observation may be the basis for comments by T. M. C. Taylor (*Pacific Northwest ferns and their allies,* University of Toronto Press, Toronto, 1970) and by D. Lellinger (*A field manual of the ferns & fern-allies of the United States & Canada,* Smithsonian Institution Press, Washington, DC, 1985) that this fern ranges south to California. Warren H. Wagner (Flora of North America Editorial Committee eds., *Flora of North America north of Mexico,* Oxford Univ. Press, New York, 1993) reported the species from every state except California, and the current find means that rattlesnake fern is now known from every mainland U.S. state.

The Salmon Mountains, which are part of the Klamath Ranges of California, are already well known for their botanical interest. The *B. virginianum* site is within 8 km of Little Duck Lake and by a tributary of French Creek. These locations are noted for their diversity of conifers, including disjunct southern populations of *Picea engelmannii* and *Abies lasiocarpa,* which are common well to the north. It is probably no coincidence that *B. virginianum,* which in the West also prefers the cooler, wetter climates of the northern states, is able

FIG. 1. A *Botrychium virginianum* plant photographed in the field in California on June 23, 1996.

to survive in California at this same southern outpost in Siskiyou County. Finally, the occurrence of *B. multifidum* and *B. virginianum* at the same site supports David H. Wagner's observation that *B. multifidum* usually occurs, "Anywhere any other *Botrychium* occurs, with the exception of *B. pumicola*" (Guide to the species of *Botrychium* in Oregon, unpubl. manuscript, University Herbarium, Dept. of Biology, University of Oregon, Eugene). *Botrychium multifidum* is relatively widespread in California, and is known from Jackson and Josephine Counties in southern Oregon. However, it too is not reported from California's Klamath Range region (KR) by Warren H. Wagner in the Jepson Manual (J. C. Hickman, ed., University of California Press, Berkeley, 1993).

I thank Alan R. Smith for useful conversations and encouragement, and acknowledge the help of my companions Doug and Maren Bell, who visited the Salmon Mountains with me. I thank Peter Zika for refereeing this note and providing additional information about the distribution of *Botrychium* species in Oregon.—JOHN C. GAME, c/o The University Herbarium, University of California, 1001 Valley Life Sciences Building, #2465, Berkeley, CA 94720-2465.

American Fern Journal 87(4):153–154 (1997)

REVIEWS

The Ferns of Tasmania: Their Ecology and Distribution, by Michael Garrett. 1996. Tasmanian Forest Research Council, Inc., GPO Box 207B, Hobart, Tasmania 7001, Australia. ISBN 0-7246-3519-X. A$50.00 (+ packing and postage at A$25 copy to Europe, Africa, and the Americas, A$20 elsewhere outside Australia). ix, 217 pp., paperbound.

Tasmania, an island about 150 miles long and wide, lies below eastern Australia at a latitude of about 41–43°S, parallel with the northern half of the South Island of New Zealand and about the same distance from the equator as Boston or northern Spain. The climate is temperate maritime, with mild winters and cool summers. About half the island is forested, and lakes are abundant. The low, mountainous terrain produces differences in rainfall and a variety of soils, which assure a wide variety of habitats for pteridophytes. Moist forests harbor about half the 101 pteridophyte species known for the island, with dry forests and alpine areas having most of the others.

The general information that begins the book includes material on fern structure and life cycle, propagation and cultivation, a checklist, and ecology, distribution, and conservation status of the pteridophytes. Details are given in three appendices. The species are listed alphabetically, one to a page, with scientific and common names, notes (especially regarding habitats), and a distribution map of 10 km^2 grid squares. Sight records are distinguished from occurrences based on herbarium vouchers. There are no descriptions. Each species has a separate color habit photograph or medium close-up that is sharp and quite diagnostic. Scattered among the habit photographs are depictions of the various habitats. A glossary, field keys to the genera and species, references, and an index conclude the volume. As a herbarium taxonomist, I found the field keys particularly interesting, for they often mention characters that one can appreciate best in the field, such as texture, frond habit, habitat, intra-Tasmanian distribution, and abundance.

As its title implies, this volume is more broadly focused than most pteridophyte Floras. It will be as valuable to ecologists and horticulturists as it is to botanists. The book is well produced and will fit easily in a backpack, although it is quite heavy. A hard cover edition is also available at A$75.00. The U.S. dollar price is currently about ¾ of the Australian dollar price. The habitat photographs and the field keys make one want to have a first-hand look at the terrain and its pteridophytes. But watch out for the "mean nakes." Tasmania is reported to have something like seven of the ten most poisonous serpent species in the world.—DAVID B. LELLINGER, U. S. National Herbarium MRC-166, Smithsonian Institution, Washington, DC 20560.

Pteridology in Perspective, edited by J. M. Camus, M. Gibby, and R. J. Johns. 1996. Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, Great Britain. ISBN 1-900347-09-1. £60. xx, 700 pp. Hardcover.

On 17–21 July, 1995, more than 100 of the world's pteridologists gathered in England for a symposium to honor one of the greatest of twentieth century pteridologists, Richard Eric Holttum (1895–1990). These meetings resulted in an impressive proceedings volume that in many ways reflects the state of present knowledge of ferns and fern allies.

In addition to introductory and symposium summary papers, as well as a section on the life and contributions of Dr. Holttum, the book is divided into six main sections, reflecting groups of presentations on similar topics that were sessions at the meetings. Within each section, the papers range from broad reviews to narrowly focused studies of individual species or groups. There is something here to stimulate and inform almost everyone with an interest in any aspect of pteridology, including floristics, conservation, biogeography, ecology, horticulture, anatomy, physiology, and of course, a variety of topics relating to classification and systematics.

Standouts among the many fine papers include Roos' summary of the state of pteridophyte floristics, Hennipman's account of problems with fern classification, Collinson's review of paleopteridology, Rothwell's summary of the paleobotanical aspects of fern phylogeny, and Stevenson and Loconte's study of higher level fern phylogeny. In particular, Haufler's exploration of species concepts and speciation in pteridophytes should be required reading for any introductory plant systematics class and apparently will lead to a symposium on the topic at the next International Botanical Congress.

My main disappointment with these proceedings is that a number of the contributors chose to submit only abstracts to the volume, rather than full papers, and that some of the papers read as though they are abridgments of research to be published more fully elsewhere. The abstracted presentations are not differentiated from the real papers in the Table of Contents, so the reader faces potential disappointment when a particular title captures his or her interest. On the other hand, if every contributor had submitted full papers, the contents might not have fit into a single volume.

Purchasers of the volume should also be wary of missing pages. My review copy had blank pages sprinkled throughout, mostly in the bibliographic sections at the ends of papers. Otherwise, the volume seems cleanly edited and is nicely bound. If it indeed is viewed as a reflection on the state of fern knowledge, then pteridologists may be proud of their achievements during the past few decades. In many cases, however, the book also provides clear indications of areas that still urgently require further research. This important summary of pteridology belongs on the bookshelf of every student of these plants.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

American Fern Journal 87(4):155 (1997)

Referees for 1997

Maintaining the quality of papers published in the American Fern Journal is perhaps one of the most difficult tasks the editor faces. Submissions for the 1997 volume covered a diversity of topics. The willingness of the following referees to undertake detailed reviews of the often challenging manuscripts helped to maintain the journal's high standards of scientific and journalistic excellence. The American Fern Society is indebted to these individuals for their service, as is the editor.—GEORGE YATSKIEVYCH.

ROBERT S. BOYD
GERALD J. GASTONY
ROY GEREAU
ROBERT G. HAMILTON
CHRISTOPHER H. HAUFLER
R. JAMES HICKEY
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MICHAEL D. WINDHAM
KEN YOUNG
PETER F. ZIKA

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American Fern Journal

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American Fern Journal 88(1):1–16 (1998)

Architecture of the Spermatozoid of *Selaginella australiensis*

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ABSTRACT.—Ultrastructural details of the mature pre-released spermatozoid of *Selaginella australiensis* are presented with a three-dimensional model based on these data. This sperm cell is highly elongated and coils for nearly three revolutions. A parallel band of spline microtubules runs around the periphery of the cell and forms the structural framework along which the four organelles are aligned. Numbers of microtubules in the spline begin with one at the tip of the cell and progressively increase to 19 toward the cell posterior. The locomotory apparatus consists of two staggered monomorphic basal bodies, referred to as the anterior and posterior basal bodies, and their corresponding axonemes. Typically, no remnant of the lamellar strip is visible in the mature spermatozoid. The anterior mitochondrion occupies over 1½ gyres at the front of the cell and as such is the largest organelle. It is inserted immediately behind the anterior basal body and underlies the posterior basal body/axoneme for over one revolution. The posterior axoneme is retained within the cell body over the spline and anterior mitochondrion. The nucleus extends approximately one gyre and is situated between the anterior mitochondrion and a smaller posterior mitochondrion. At the rear of the cell is a plastid that contains two large juxtaposed starch grains. Similarities of this cell with other lycophytes include the staggered monomorphic basal bodies and increase in spline microtubules from front to back. Commonalities with bryophyte and charalean spermatozoids include cellular organization and shape, and the lack of a lamellar strip in the mature cell.

Selaginella is a key taxon in understanding the early events in land plant diversification (DiMichele and Skog, 1992). The fossil record of this phylogenetically significant genus dates back to the Carboniferous, making *Selaginella* second only to *Lycopodium* in geological age (Bierhorst, 1971). Living representation of this lycopsid is extremely diverse, with over 700 species worldwide (Webster, 1992; Valdespino, 1993). Yet, the precise placement of *Selaginella* in the plant kingdom is unclear. The paucity of detailed comparative studies on anatomical and ultrastructural features of lycopsids, and especially the ligulate taxa, has confounded resolution of relationships among these basal plant lineages (Garbary and Renzaglia, in press). Clearly, additional studies on this taxonomically complicated and phylogenetically crucial genus are warranted.

Spermatogenesis has proven to be a rich source of phylogenetic information (Garbary et al., 1993; Mishler et al., 1994; Duncan et al., 1997; Maden et al., 1997). The major limitations in using male gametogenesis for phylogenetic reconstructions are the low numbers of organisms that have been investigated and the incomplete data sets for those taxa that have been observed. In bryophytes, much attention has been placed on elucidating the microanatomy of

the locomotory apparatus or blepharoplast (Carothers and Rushing, 1988; Renzaglia and Duckett, 1991). Few studies of developmental processes and mature cell structure have been undertaken (Vaughn and Renzaglia, in press). In pteridophytes, even fewer organisms and structural features have been documented than in bryophytes (Duckett 1973; 1975; Duckett and Bell, 1977).

Ultrastructural observations of *Selaginella* spermatogenesis is limited to one species, *S. kraussiana* (Kunze) A. Braun. In gross morphological features, this spermatozoid bears an uncanny resemblance to those of bryophytes. Although an idealized reconstruction of the mature cell for this species was presented by Robert (1974), many details of the cellular organization remain unclear. Thus, we undertook an ultrastructural investigation of the mature sperm cell of a second species of *Selaginella, S. australiensis* Baker. Descriptions of precise structural features and a three-dimensional reconstruction of the cell based on these data are presented. Comparisons are made with motile gametes from a wide range of green plants, with emphasis placed on identifying similarities and differences among biflagellated gametes of *Selaginella, Lycopodium* sensu lato, bryophytes and Charales.

Materials and Methods

Selaginella australiensis was collected in the rainforest on Mount Wilson, Blue Mountains, New South Wales, Australia, in October, 1991. Plants were maintained in the greenhouse and monitored for sporangial production. Mature microsporangia were dissected and placed on filter paper moistened with distilled water in petri plates. The plates were sealed with parafilm and placed in an incubator at 20°C with a 12 hr light/dark cycle. After 10 days, the cultures were examined daily until swimming sperm cells were detected. Because of differing rates of microgametophyte development among the several microsporangia per plate, cultures harvested at the first sign of mature spermatozoids generally contained all stages of spermatogenesis.

Microsporangia were lifted from the filter paper and placed in microfuge tubes containing 6% glutaraldehyde in 0.05 M Pipes buffer (pH 7.4). Microspores were liberated from sporangia and gently cracked with the end of a flame-sealed Pasteur pipet. Without mechanical disruption of spore walls, fixative and resin will not penetrate the microgametophyte. After immersion in the primary fixative for 2–4 hr, the microspores were washed four times in 0.1 M cacodylate buffer (pH 7.2) and post fixed in 2% OsO_4 in the same buffer for 2 hr. Prior to withdrawing solutions, the spores were pelleted in a microcentrifuge for 30 sec. After washing with distilled water, the spores were "en bloc" stained in 1.0% aqueous uranyl acetate for 16 hr at 4°C. The specimens were rinsed in distilled water, dehydrated in a graded acetone series, replaced by propylene oxide, infiltrated slowly over approximately one week with 1:1 Spurrs/Embed 812 resin, embedded and cured at 60°C for 16 hr. Thin sections were collected on copper grids and post-stained with 2% uranyl acetate in ethanol and basic lead citrate for five min each. Grids were observed on a Philips 201 or Hitachi 7100 TEM.

All micrographs of cross sections are oriented as if viewing from the anterior of the cell, i.e., from the front of the MLS. In such an orientation, the triplet and double microtubules in basal bodies and flagella overlap in a counterclockwise direction. References to right and left in the text refer to this orientation.

RESULTS

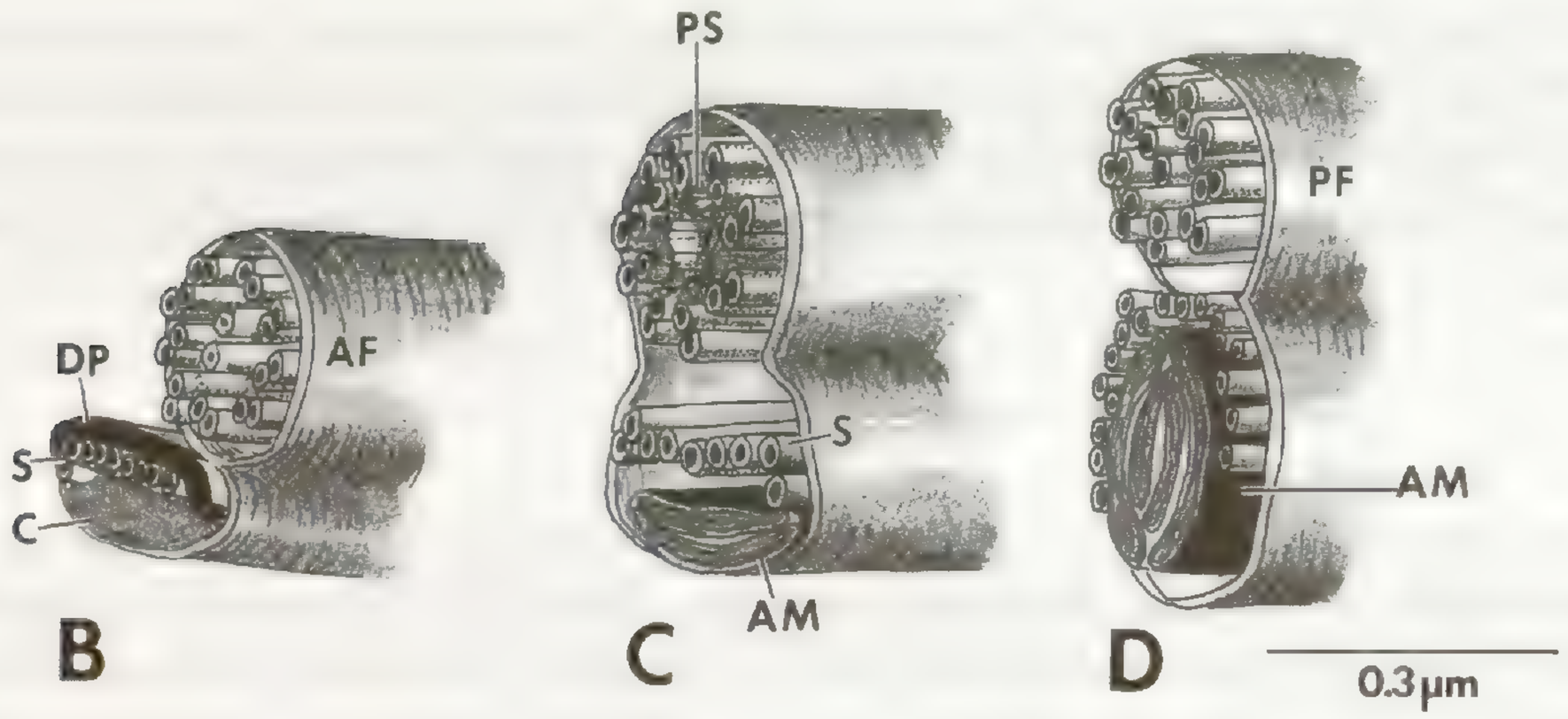
The mature sperm cell of *Selaginella australiensis* is biflagellated, streamlined, and coiled for nearly three revolutions (Figs. 1A, 26). A spline composed of a band of parallel microtubules (MTs) runs longitudinally around the periphery of the cell. The four organelles are aligned along the spline and consist of a highly elongated anterior mitochondrion (AM), a central cylindrical nucleus, a small posterior mitochondrion (PM) and a posterior plastid with two large starch grains. From front to back, there is a gradual increase in cross sectional diameter of the organelles (Figs. 1A, 26). The two apical flagella are directed toward the cell posterior and are highly staggered in insertion. The extreme anterior of the cell exhibits a right-hand curve such that it is directed inward, toward the remaining coils (Fig. 1A).

The spline begins with a single microtubule on the dorsal side of a flattened anterior cytoplasmic remnant (Figs. 2, 3, 21). From this region, MTs progressively increase in number toward the cell posterior up to a maximum of 19 where they overlie the plastid (Figs. 1, 3–20, 24). Additional MTs that run more or less parallel to the cell axis are scattered in the anterior cytoplasm (Figs. 6, 8, 9, 23).

The anterior basal body (ABB) is inserted on the right-hand side of the anterior-most portion of the cell where it overlies a spline of 5 or 6 MTs (Fig. 6). This basal body is compacted, tapered toward the front, and the MT triplets are occluded by an electron opaque matrix (Figs. 6, 21, 27). A dorsally projecting ridge begins above the spline where it contains two MTs (Fig. 4) and extends over the upper left-hand side of the ABB and anterior transition region of the flagellum, including the stellate pattern (Figs. 5–8). A single MT or tubular structure appears to extends the length of the ridge (Figs. 4, 5, 21) but it is normally obscured by the electron-opaque material that fills the ridge. From the beginning of the ridge to the ABB transition region (Figs. 7, 8), the underlying spline increases from two to eight MTs.

There is no overlap between the ABB and posterior basal body (PBB); the PBB is inserted (Fig. 11) directly behind the point of emergence of the anterior axoneme (Figs. 1A, 9, 10) which diverges to the right of the cell (Figs. 11–16). The PBB is positioned directly in the center of the cell over the spline and AM (Figs. 11–16). This basal body resembles the ABB in that it is occluded and constricted anteriorly (Figs. 22 and 23). A dense plaque overlies the spline along the length of the ABB and anterior stellate pattern, up to the insertion of the PBB (Figs. 5–9, 21–23, 27).

In the nearly mature spermatozoid the lamellar strip (LS) extends from the cell anterior to the back of the PBB (Fig. 23). In the final stages of differenti-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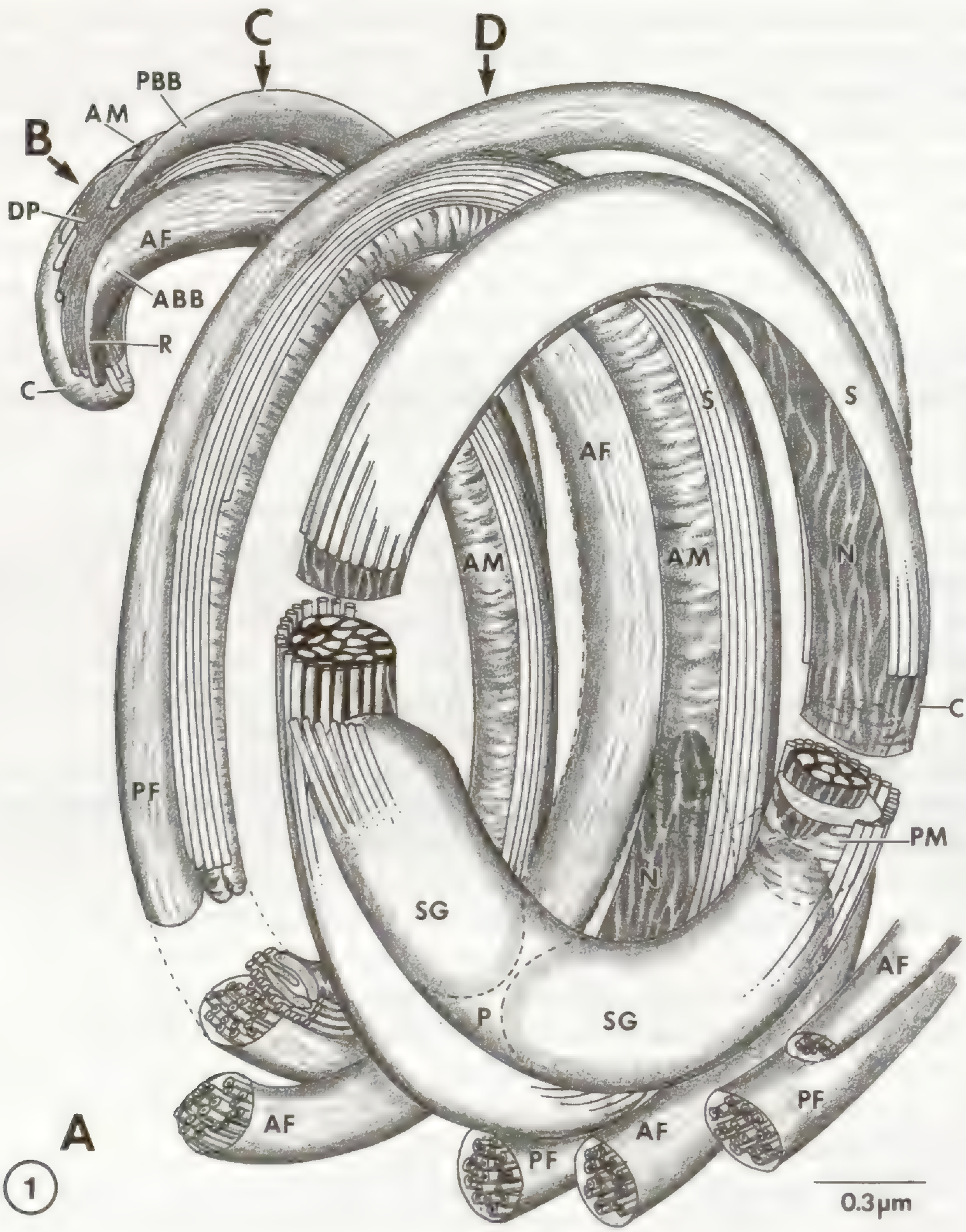
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ation, the LS disintegrates at equal rates along its length. Only rarely is this component of the multilayered structure still visible in the mature cell (Figs. 5, 23).

The transition regions of both BBs contain well-defined stellate patterns (Figs. 7, 8, 12, 21, 22) that are located within the main body of the cell, i.e., they do not or only slightly extend into the flagellar shaft. Faint (Fig. 7) and dense (Figs. 8, 12) alternating profiles characterize the stellate patterns such that in longitudinal sections they appear as broken lines (Figs. 21, 22). The length of both stellate patterns ranges from 150–200 nm.

The anterior mitochondrion (AM) is the longest organelle in the cell; it extends ca. 1½ gyres, over half the length of the cell (Figs. 1A, 11–18, 26). The AM commences slightly in front of (Fig. 22) or beneath the PBB (Fig. 11). At this level the AM is lens-shaped in cross section, devoid of cristae, and overlain by nine MTs (Figs. 1B, 11). Two or three MTs wrap around the right-hand side of the AM. These MTs are connected by dense fibers to the plasma membrane (Figs. 11, 12). Folds of the cristae are first visible in the AM at least ½ gyre from the cell anterior in a region where the spline consists of ten MTs and underlies the posterior flagellum (Figs. 13, 26). The AM exhibits increasingly baffled cristae as it progresses toward the posterior of the cell (Figs. 13–17). For the first half revolution, the cristae folds are oriented horizontally to the cell axis (Figs. 1C, 13, 14). As the folds increase in number, their orientation rotates to a vertical position (Figs. 1D, 16, 17). MTs are added primarily to the left-hand side of the spline, up to 18 at the posterior of the AM. They wrap around both sides of the AM. Typically three or four MTs are associated with the plasma membrane on the right side, whereas the number of MTs increases from two to nine on the left-hand margin of the AM. The MTs on both sides of the AM are attached to the plasmalemma by dense connectors (Figs. 14–17).

The posterior flagellum is intimately associated with the spline and is embedded in the cell for most of the length of the AM (Figs. 13–17, 26). It emerges from the cell ca. one revolution behind the anterior flagellum (Figs. 1A, 26) and it extends nearly one gyre around the cell beyond the anterior flagellum (Fig. 26). In comparison to the individual profiles of the cell, the flagella are relatively large and in the anterior region are almost as wide as the cell itself (Figs. 11–15).

The nucleus is cylindrical and coils for approximately one revolution (Figs. 1A, 26, 28). It is pointed on either end where it overlaps the two mitochondria,

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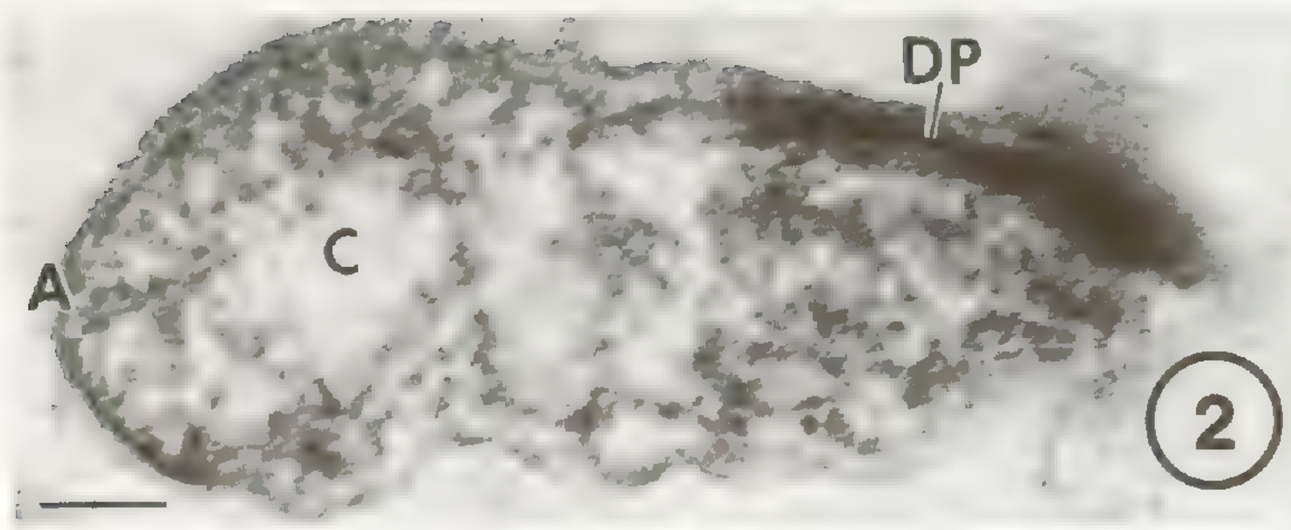
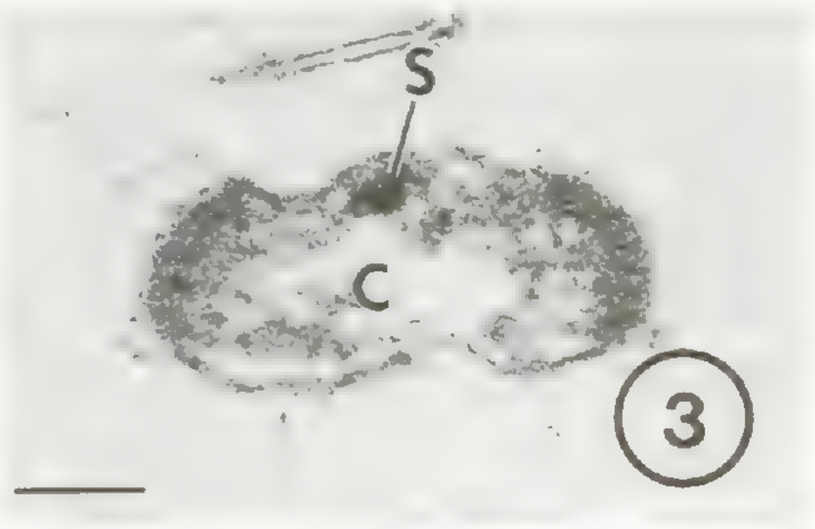
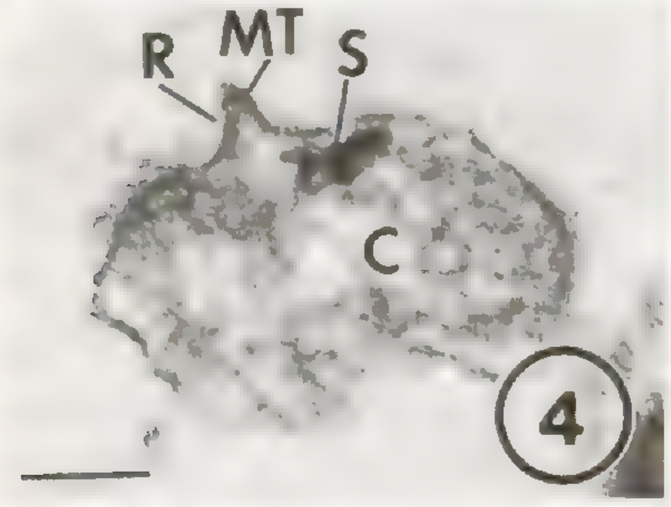
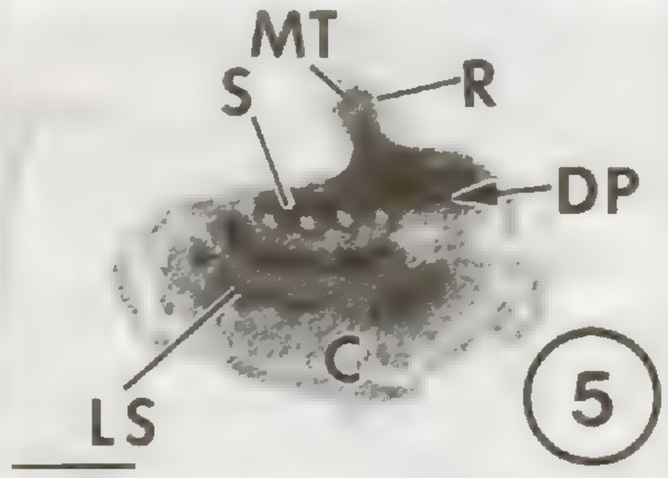
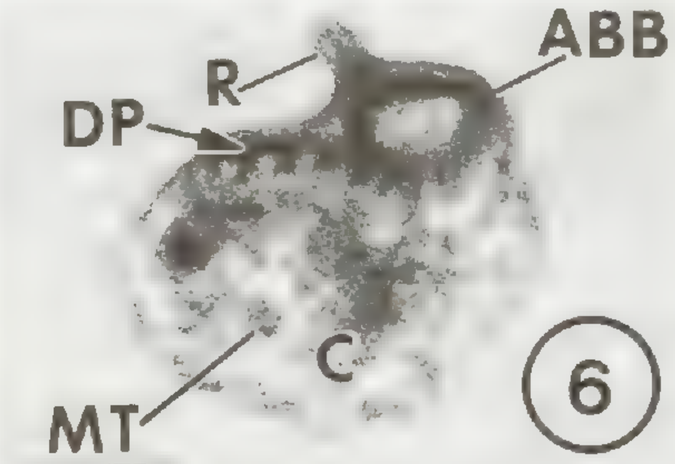
FIG. 1. Reconstructions of the spermatozoid of *Selaginella australiensis*. A) whole cell, plasmalemma omitted from illustration. B, C, D) cut out enlargements (illustrated with plasmalemma) at the levels labeled B, C, and D, respectively, on Fig. 1A. Abbreviations: A=anterior of cell, ABB=anterior basal body, AF=anterior flagella, AM=anterior mitochondrion, AS=anterior stellate pattern, BB=basal body, C=cytoplasm, CM=cytoplasmic mass, DP=dense plaque, F=flagellum, LS=lamellar strip, MT=microtubule, N=nucleus, P=plastid, PBB=posterior basal body, PF=posterior flagellum, PM=posterior mitochondrion, PS=posterior stellate pattern, R=ridge, SG=starch grain, S=spline, V=vesicle.

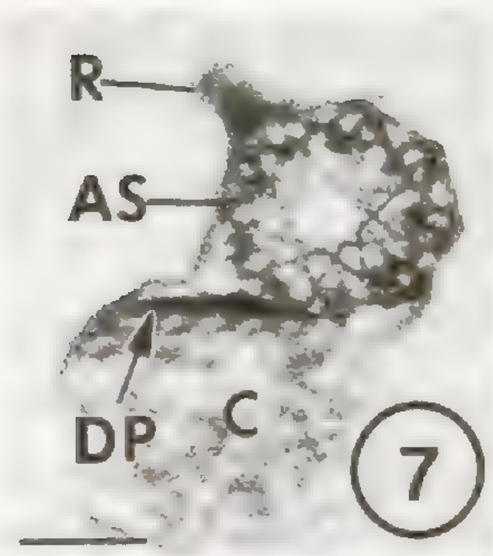


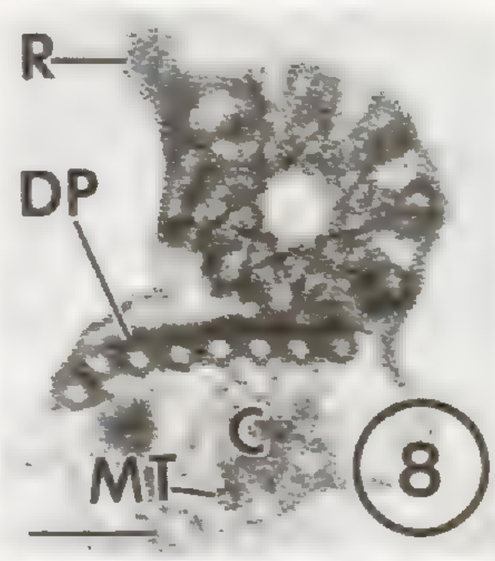


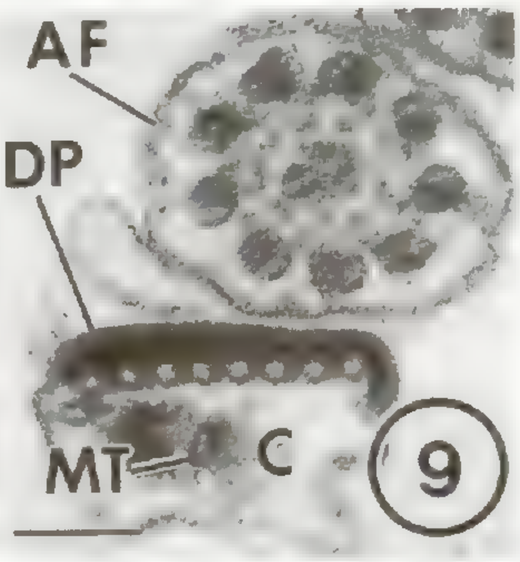


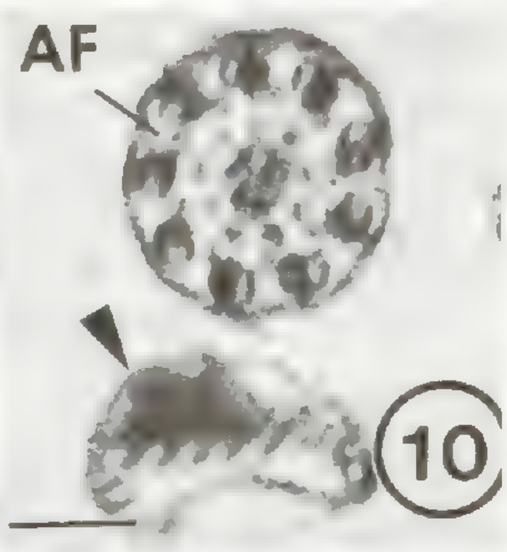


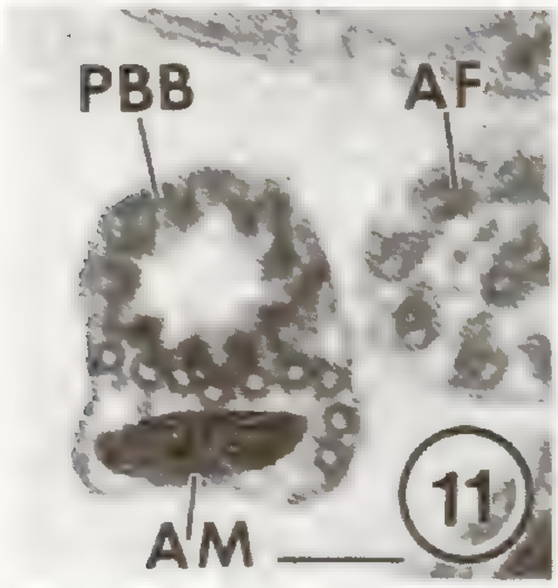


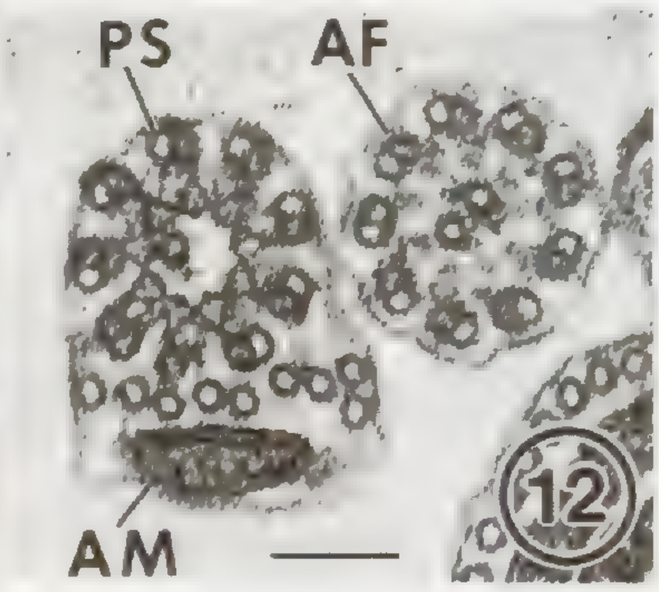


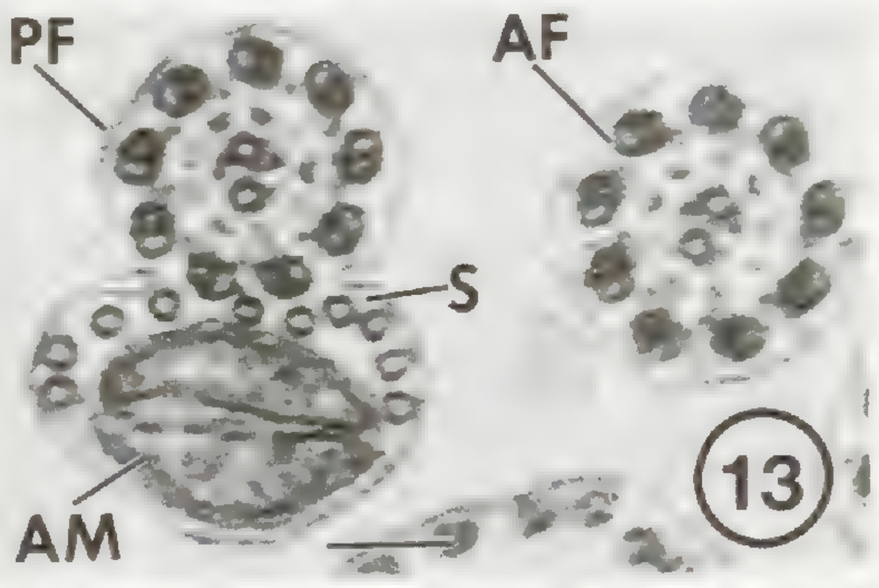


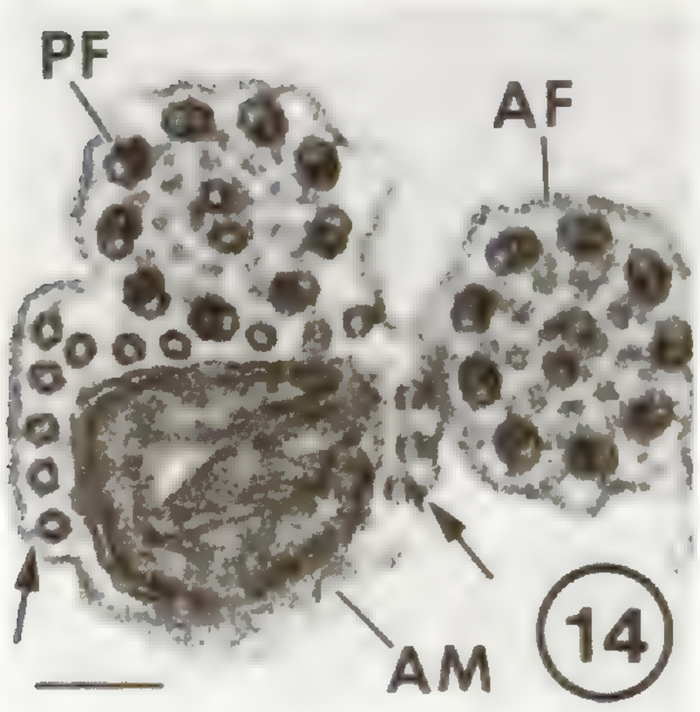


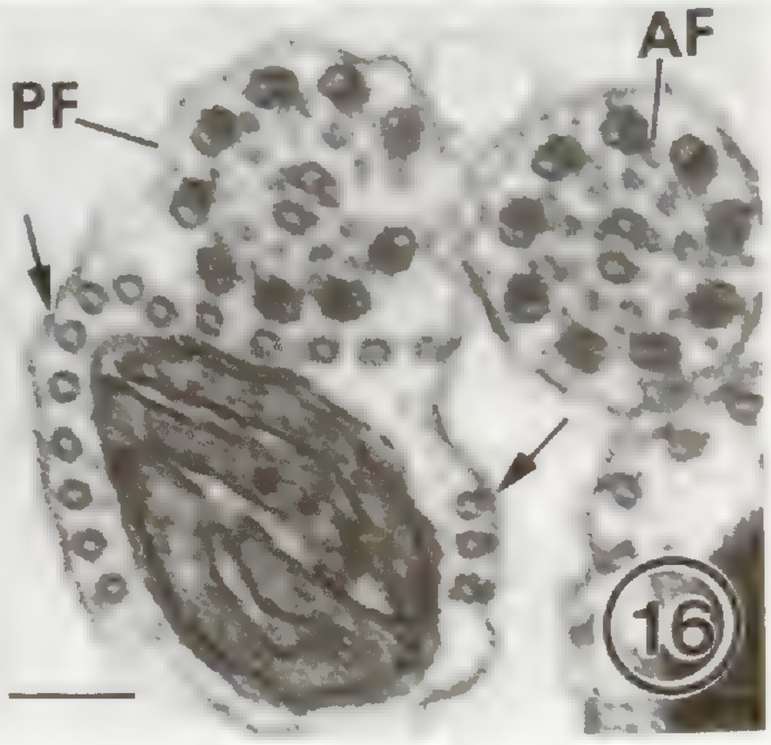












FIGS. 2–16. Electron photomicrographs documenting the reconstruction in Fig. 1. FIG. 2. Tangential section through extreme anterior of cell showing cytoplasmic remnant overlain dorsally by dense plaque. FIGS. 3–16. Series of cross sections from cell anterior toward posterior. 3) extreme anterior of cell containing cytoplasm and single, dorsally centered spline microtubule. 4) dorsal ridge containing a single microtubule projects to the left of spline containing two microtubules.

the AM and PM (Figs. 1A, 18, 20, 27, 28). At its anterior, the nucleus is surrounded dorsally and laterally by the AM (Fig. 18). Posteriorly, the PM cradles the ventral nuclear surface (Fig. 20). Typically the spline overlying the nucleus contains 18 MTs. Internally, twisted rods, surrounded by a dense matrix, extend the length of the nucleus (Figs. 19, 28).

The PM is wedged between the nucleus and plastid, overlapping the latter only slightly (Figs 1A, 25, 27, 28). This organelle contains densely folded cristae (Fig. 27).

The plastid terminates the cell and contains two large starch grains oriented end to end (Figs. 1A, 25). Of all the organelles, the plastid has the widest cross sectional diameter (Fig. 26). The 18 or 19 spline MTs encasing the plastid are widely separated and are splayed among a scalloped membrane system (Fig. 24).

Deletion of the bulk of the cytoplasm occurs in the final stages of cellular differentiation. This process involves an internal pinching off of a cytoplasmic mass along the coils from anterior to posterior (Fig. 26). When released from the antheridium and upon commencement of motility, the cell eliminates the central mass of extraneous cytoplasm. Prior to release, spermatozoids frequently show attachment of the cytoplasmic body along the posterior coil, especially along the plastid (Figs. 24–28). Abundant vesicles that surround the plastid may function in elimination of cytoplasm and likely contribute to the scal-

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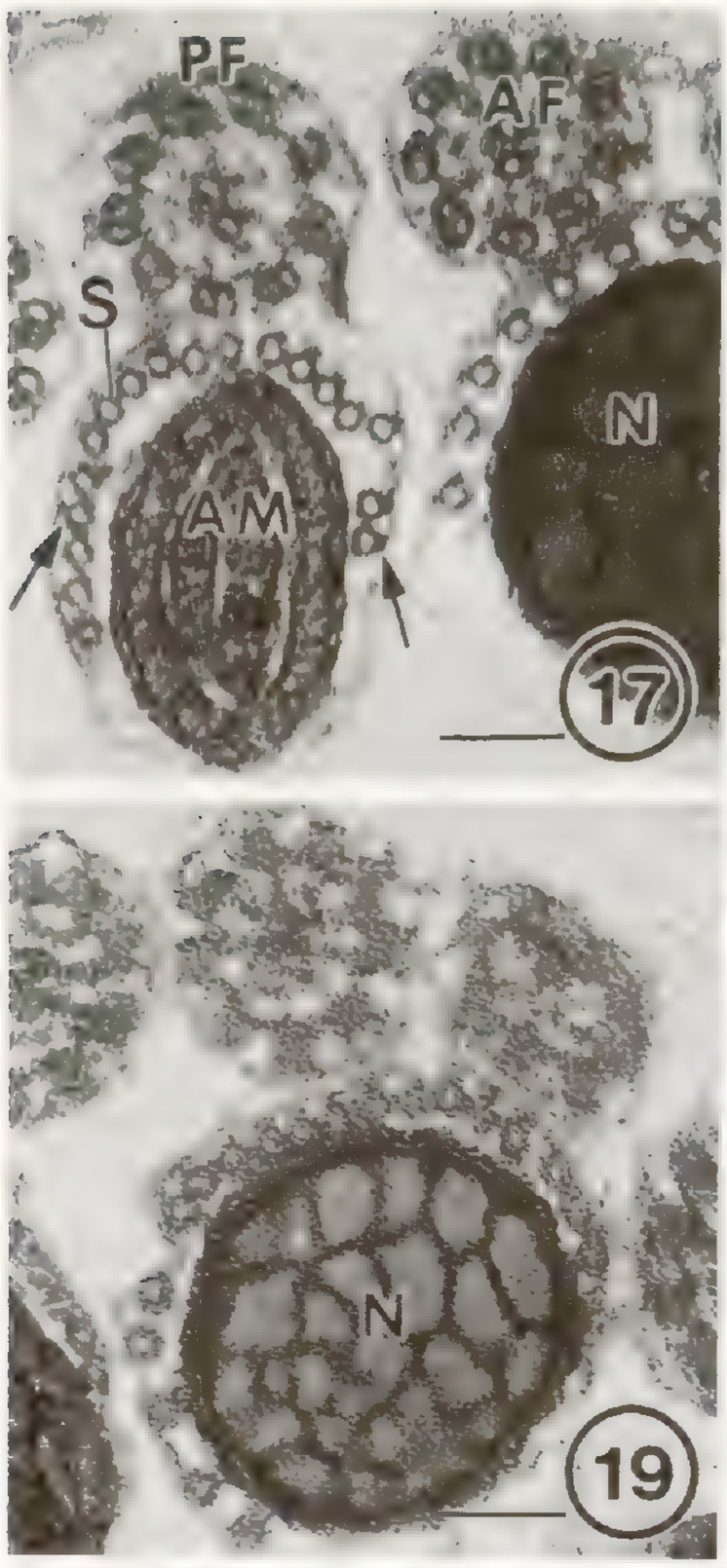
5) dorsal ridge, containing electron opaque matrix and single microtubule, and dense plaque overlie four spline microtubules and remnant of lamellar strip. 6) constricted anterior basal body with occluded microtubule triplets and overlying ridge is positioned on the right side of spline with five microtubules; note stray microtubules in cytoplasmic remnant and dense plaque over spline. 7) faint anterior stellate pattern with ridge containing electron opaque material overlie six spline microtubules and cytoplasmic remnant. 8) anterior stellate pattern and dorsal ridge with single microtubule over eight spline microtubules and dense plaque; note stray microtubule in cytoplasmic remnant. 9) anterior flagellum has emerged from cell body; cytoplasm is overlain by nine spline microtubules and dense plaque; stray microtubule in cytoplasmic remnant. 10) dense material at tip of posterior basal body (arrowhead), overlying nine spline microtubules; anterior flagellum separate from cell body. 11) posterior basal body over lens-shaped anterior mitochondrion, overlain and wrapped to the right side by nine spline microtubules; anterior flagellum to right-hand side of cell. 12) anterior mitochondrion overlain by nine spline microtubules and posterior stellate pattern; anterior flagellum to the right. 13) anterior mitochondrion displaying horizontally oriented cristae, now overlain by ten spline microtubules that wrap around both sides of the anterior mitochondrion; posterior flagellum remains inserted in cell, centered above spline; anterior flagellum to right. 14) increasingly baffled cristae in anterior mitochondrion overlain by 14 spline microtubules that wrap to either side of the anterior mitochondrion and are connected by dense material to the plasmalemma (arrows); posterior flagellum remains in the cell body and anterior flagellum diverges to the right. 15) posterior flagellum inserted above 16 spline microtubules, overlies more rounded, baffled anterior mitochondrion; microtubules on sides of anterior mitochondrion are attached to plasmalemma by dense connectors (arrows); anterior mitochondrion orientation rotated more vertically. 16) anterior mitochondrion further rotated toward vertical and flanked by 17 spline microtubules attached to plasmalemma on the sides (arrows). Posterior flagellum retained inside cell and anterior flagellum to the right. Scale bars=0.1 μm. For labels, see legend to Fig. 1.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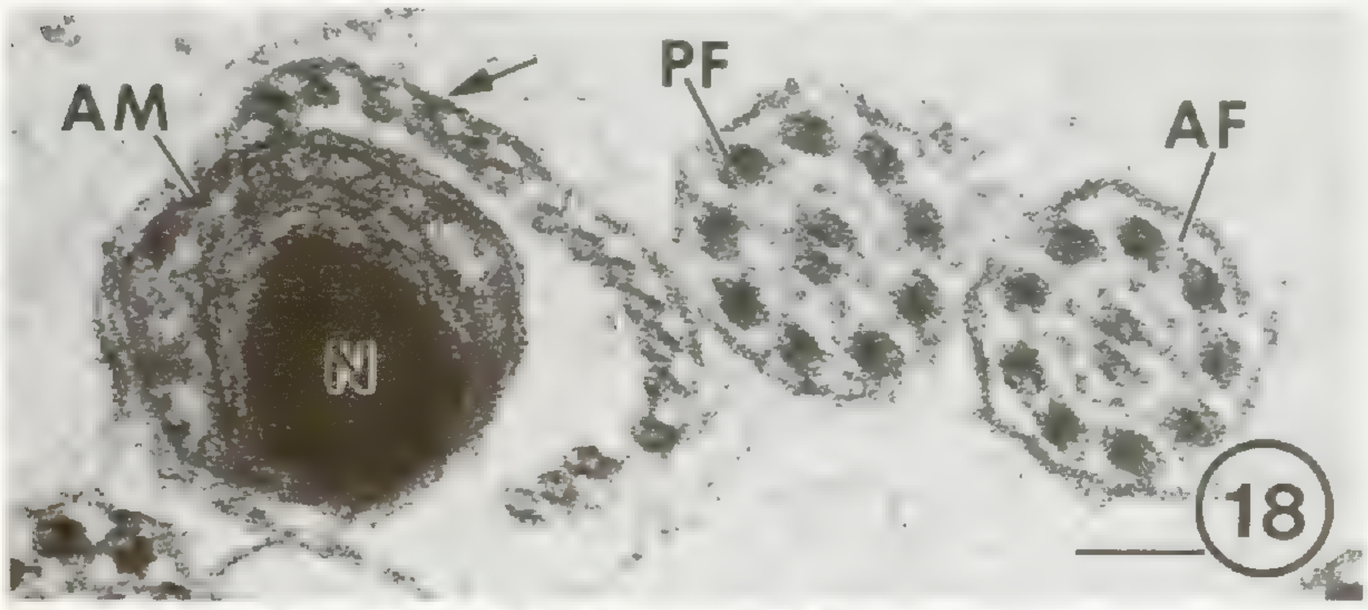
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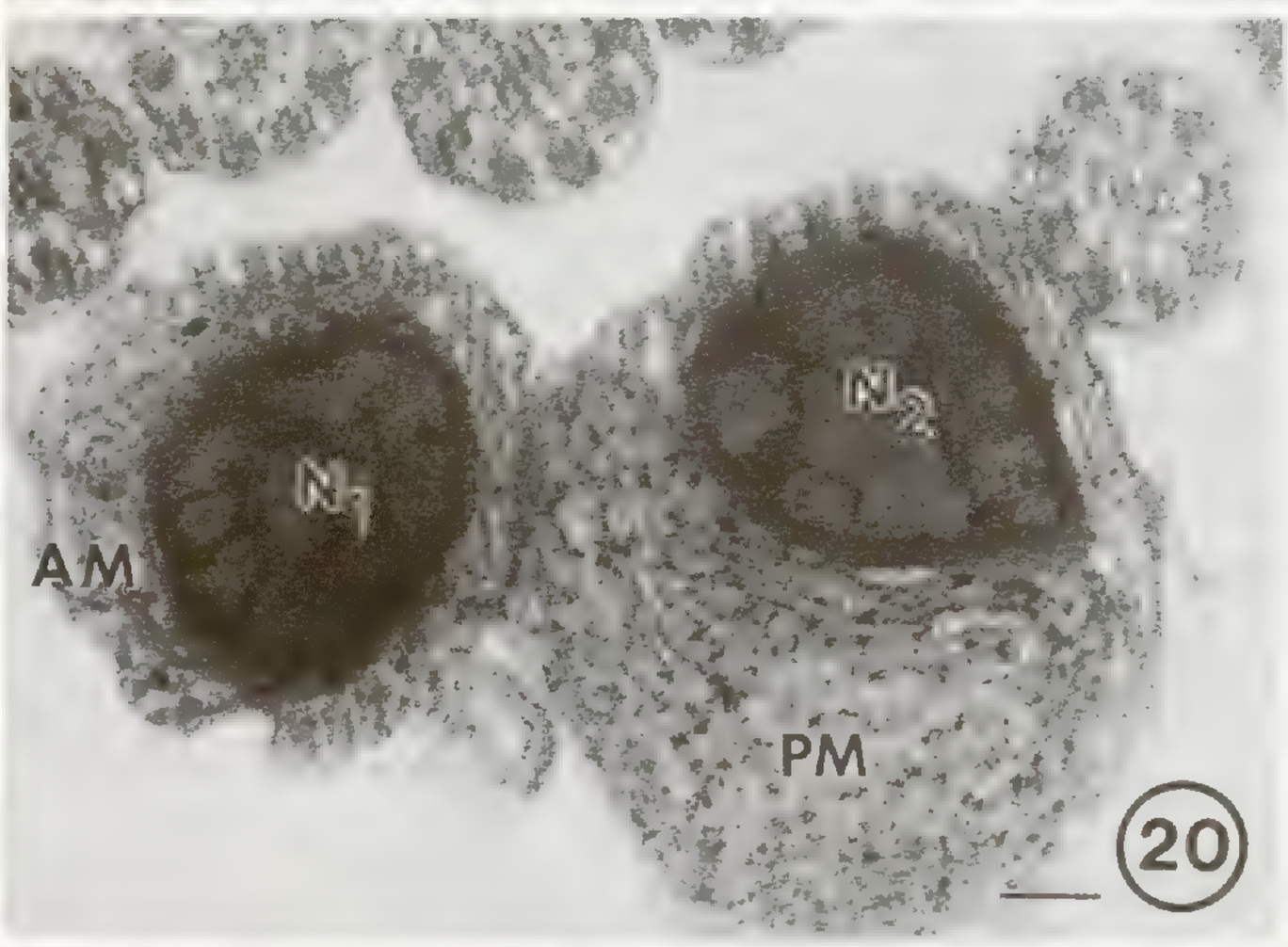
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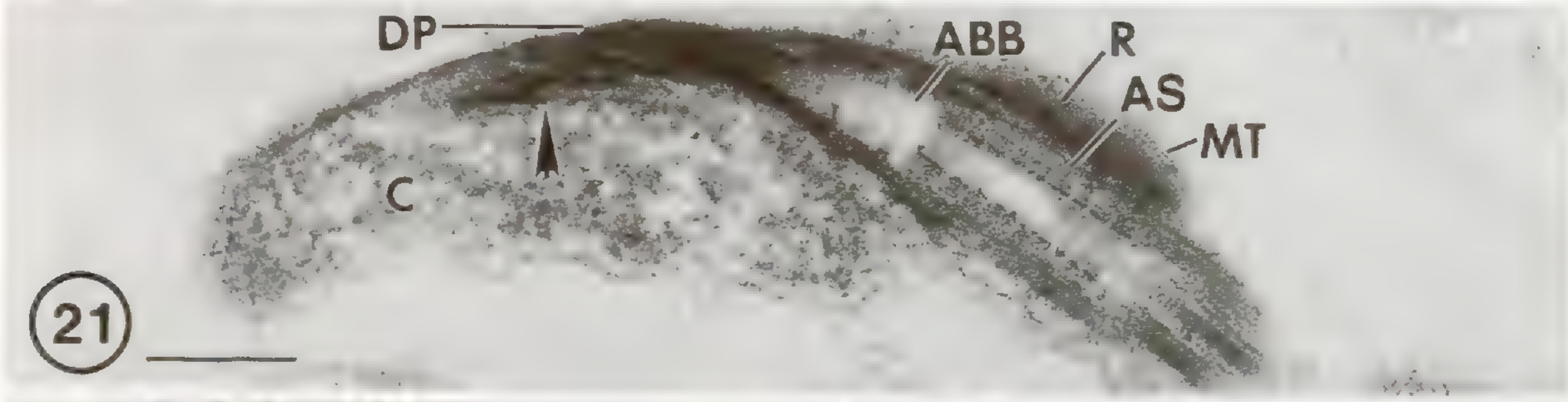
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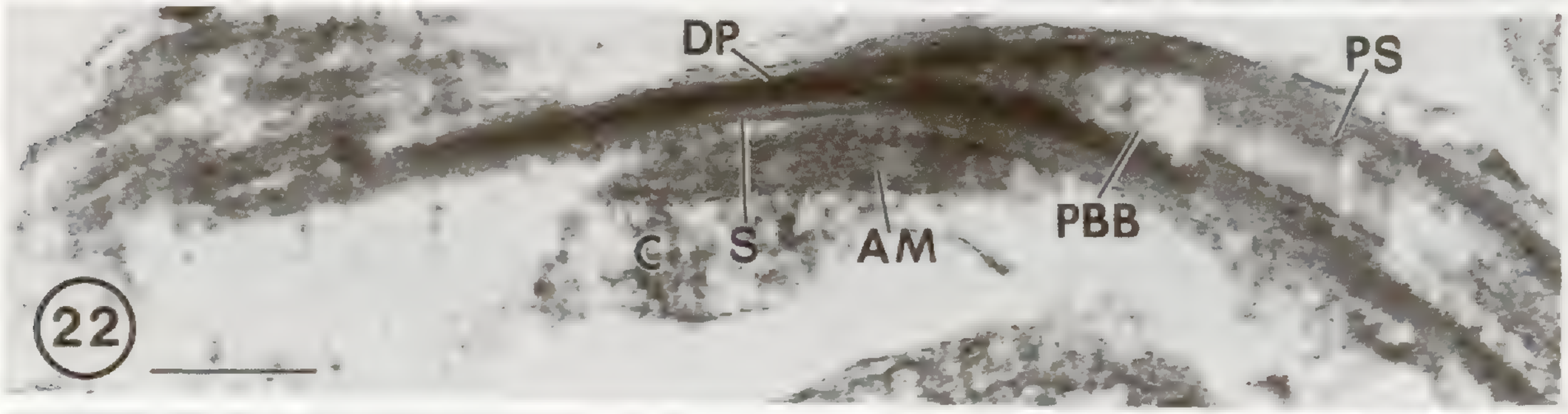
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loped membrane system that is associated with the spline in this region (Figs. 25–28).

Discussion

The spermatozoids of lycopsids are highly diverse in structure and organellar content. On the one extreme is the minute, coiled sperm cell of *Selaginella,* which contains a locomotory apparatus with two staggered flagella and four organelles aligned along a narrow spline. A curious feature of this spermatozoid is the incredible size of the anterior mitochondrion, which extends over half of the cell length. *Selaginella* appears to be unique among both plant and animal spermatozoids in having an anterior mitochondrion that is longer than the nucleus. In bryophytes, the nucleus extends most of the length of the coiled sperm cell, and cell length is highly correlated with nuclear DNA content (Renzaglia et al., 1995). Sperm cells of *Selaginella* are approximately 25.0 μm long and as such are comparable to moss spermatozoids in length. Because the nuclei in *Selaginella* do not extend the length of the spermatozoids, it is likely that DNA content in this genus is even lower than that recorded for mosses (0.38–0.92 pg; Renzaglia et al. 1995). Indeed, nuclear genomic DNA content is likely to be among the lowest in archegoniates, approximating that of the hornwort, *Notothylas* (C=0.17 pg; Renzaglia et al., 1995) and the flowering plant *Arabidopsis* (2C=0.33 pg; Arumuganathan and Earle, 1991). Undoubtedly, the volume of the extremely minute nuclei in *Selaginella* spermatozoids is markedly lower than that of other lycopsids, which have larger, broad-cylindrical to ovoid nuclei (Maden et al., 1997).

Compared to *Selaginella,* greater complexity characterizes spermatozoids of *Lycopodium obscurum* L. (Maden et al., 1996; Renzaglia et al., 1994), *Lycopodiella lateralis* (R. Br.) B. Øllg. (Maden et al., 1997), and *Palhinhaea cernua*

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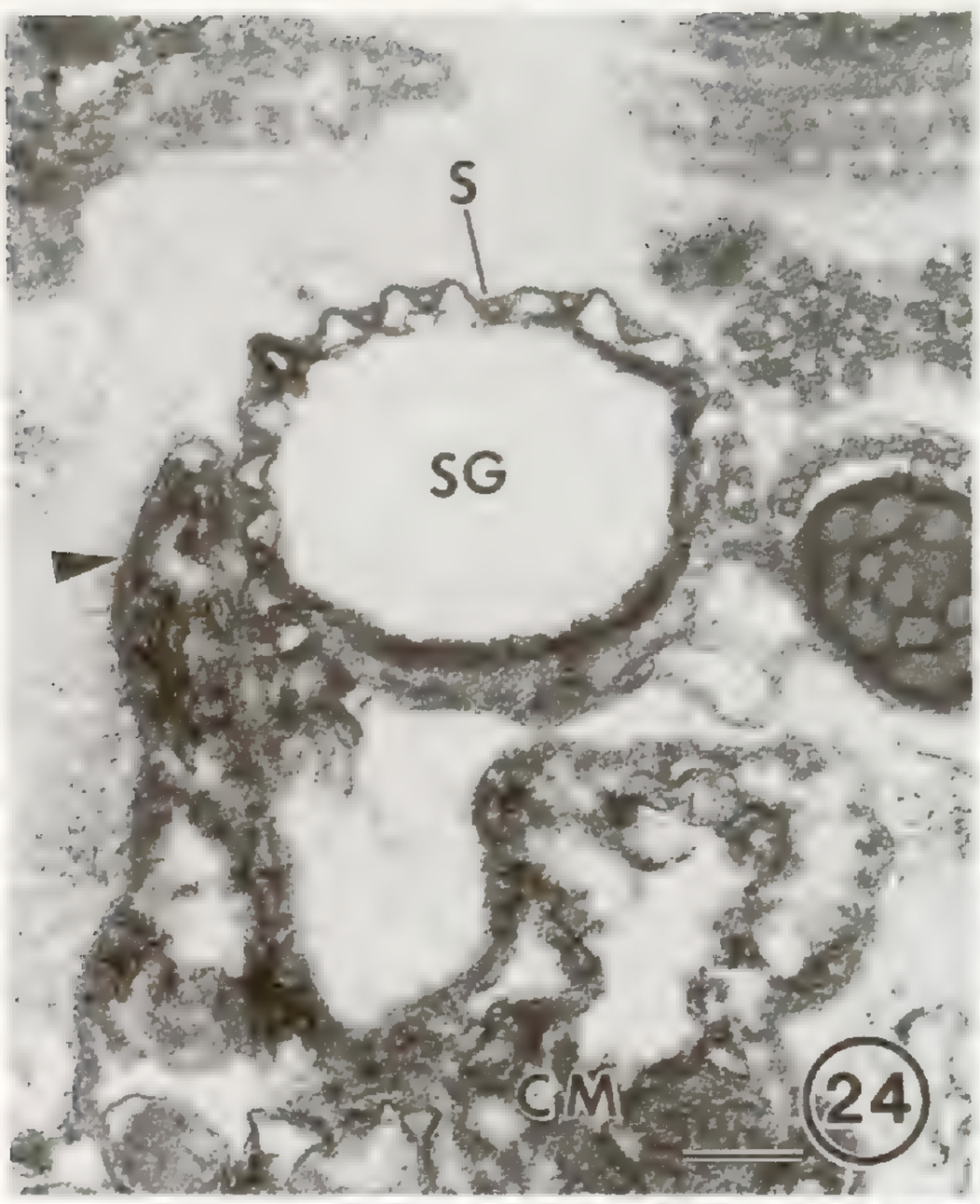
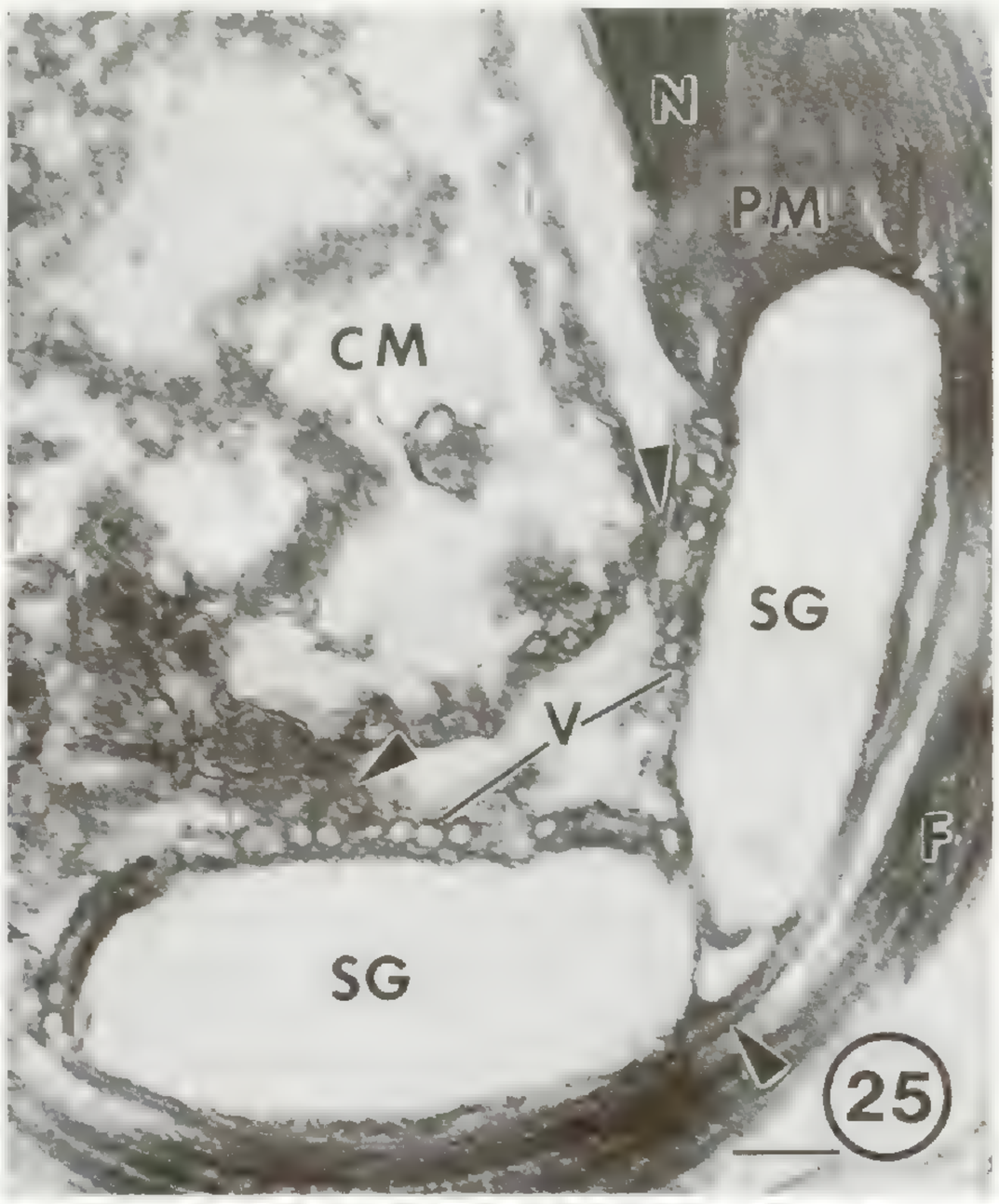
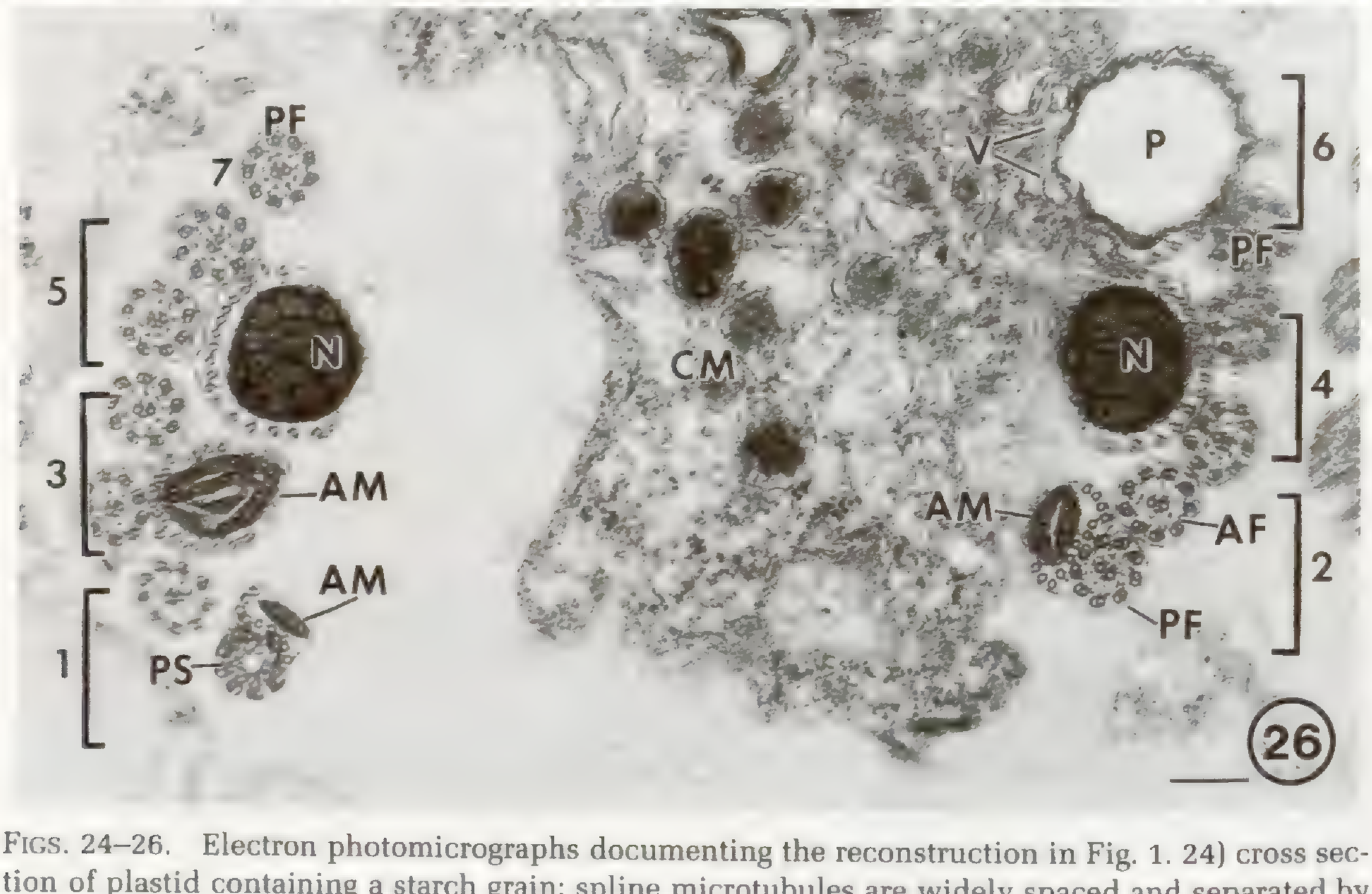
FIGS. 17–23. Electron photomicrographs documenting the reconstruction in Fig. 1. FIGS. 17–20. Progressively more posterior cross sections from back of anterior mitochondrion to end of nucleus. 17) posterior flagellum embedded in cell over spline of 18 microtubules; subtending anterior mitochondrion contains vertically aligned cristae; note connections between microtubules and plasmalemma (arrows) on both sides of anterior mitochondrion. 18) overlap between narrow anterior tip of nucleus and anterior mitochondrion; both anterior and posterior flagella emerged from cell. 19) mid-region of nucleus showing rods compacted in an electron opaque matrix. 20) adjacent profiles of anterior (N_1) and posterior (N_2) nuclear regions one revolution apart showing overlap between nucleus and anterior and posterior mitochondria. FIGS. 21–23. Longitudinal sections of cell anterior. 21) extreme anterior of cell showing cytoplasmic remnant, spline impregnated with dense matrix (arrowhead) that continues into constricted anterior basal body; broken lines of stellate pattern correspond to dark and faint regions in cross section (cf., Figs. 7, 8). 22) longitudinal section of posterior basal body overlying spline; basal body and stellate pattern as in Fig. 21; flattened anterior mitochondrion subtends spline and extends in front of posterior basal body. 23) longitudinal section showing stagger between anterior basal body and posterior basal body; anterior basal body is sectioned tangentially along its inner surface; disintegrating lamellar strip underlies spline and stray microtubules are visible in cytoplasm. Scale bars=0.1 μm. For labels, see legend to Fig. 1.







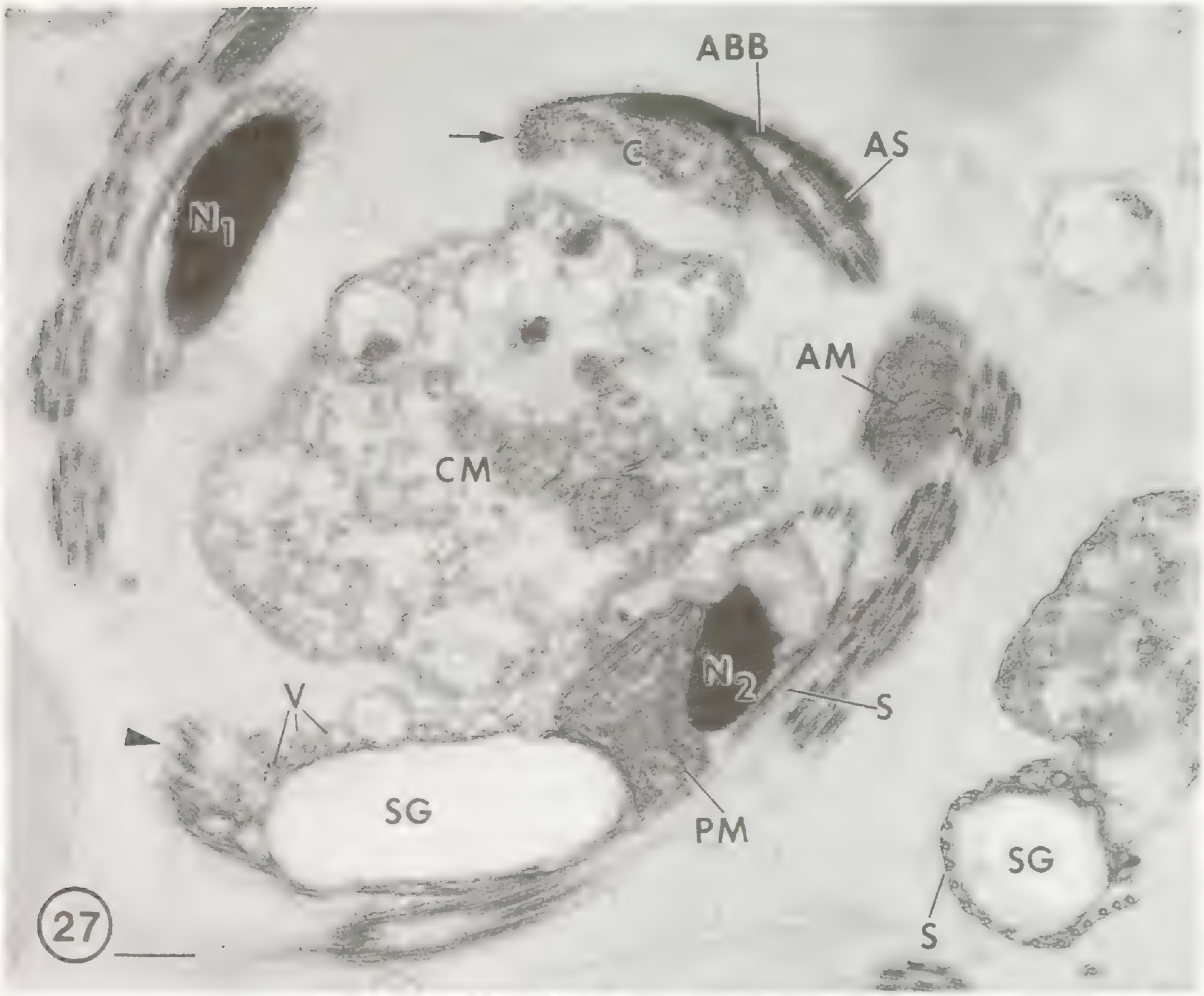
FIGS. 24–26. Electron photomicrographs documenting the reconstruction in Fig. 1. 24) cross section of plastid containing a starch grain; spline microtubules are widely spaced and separated by scalloped membrane system; cytoplasmic deletion is nearly complete, with attachment of cytoplasmic mass only on left of plastid (arrowhead). 25) longitudinal section of plastid positioned along spline microtubules (arrowhead), behind posterior mitochondrion and nucleus; two large abutting starch grains occupy plastid; numerous vesicles surround inner surface of plastid and cytoplasmic mass is attached only in limited regions (arrowheads). 26) cross section of whole cell showing successive ½ gyres (1–7); anterior mitochondrion is visible in coils 1–3, nucleus in coils 4 and 5 and plastid in coil 6; note gradual increase in coil diameter and increase in spline microtubule number; posterior flagellum is retained in cell body in coils 2 and 3 and extends beyond anterior flagellum for one revolution (coils 6 and 7). Scale bars=0.15 μm for Figs. 24, 25; 0.2 μm for Fig. 26. For labels, see legend to Fig. 1.

(L.) Vasc. & Franco (Robbins and Carothers, 1978). Sperm cells of these lycopsids have multiple organelles, especially mitochondria, more extensive cytoplasm, and wide splines (150–200 MTs in diameter). Considerable variability is evident in external architecture of these spermatozoids. In particular, the body of the sperm of *Lycopodium* is slightly elongated and coiled in an anterior-posterior orientation, whereas the sperm body of *Lycopodiella* and *Palhinhaea* is ovoid with the apical locomotory apparatus coiled laterally. Sperm cells of *Phylloglossum* are slightly coiled and similar to *Lycopodium,* except they contain approximately 20 flagella (Renzaglia, unpublished). *Isoetes* spermatozoids also are multiflagellated, but they are more streamlined and coiled than those of *Phylloglossum* (Thomas, 1976).

The work of Robert (1973, 1974, 1977) on *Selaginella kraussiana* provided the first ultrastructural descriptions of spermatogenesis in the genus. The emphasis of his studies was to outline the developmental events involved in sperm cell maturation. Although Robert (1974) presented a three-dimensional model of the swimming sperm of *Selaginella,* he included few micrographs to support his drawing. Detailed comparisons of Robert's reconstruction and Fig. 1A in the present study reveal commonalities in cellular architecture as well as significant differences. Both cells are approximately the same length and contain the same number and position of organelles. The anterior mitochondrion is extremely long and the nucleus is positioned behind this organelle between it and the posterior mitochondrion. The plastid terminates the cell and it contains two long linear starch grains (Robert, 1974, 1977). The substructure of the nucleus, plastid, and mitochondria are likewise equivalent in the two species.

The most significant difference in sperm organization between *S. kraussiana* and *S. australiensis* is the position of the posterior basal body. In *S. kraussiana,* this basal body is widely separated from the anterior basal body; it overlies the back of the anterior mitochondrion. In *S. australiensis* the posterior basal body is positioned just behind the anterior basal body at the front of the cell. The posterior flagellum remains within the main cell body, emerging from the cell approximately one revolution from the anterior flagellum. Examination of micrographs in Robert (1974) suggests that, although not described, this feature may also exist in *S. kraussiana.* Robert describes a hook in the back of the spermatozoid of *S. kraussiana* at the position of the posterior mitochondrion. No such bend is evident in the spermatozoid of *S. australiensis.*

Among other archegoniates, sperm cells of *Selaginella* are most similar to those of bryophytes. In mosses, liverworts, and hornworts, spermatozoids are streamlined, highly coiled, biflagellated, and contain four organelles in the same arrangement along a microtubular framework (Renzaglia and Duckett, 1988). *Selaginella* also shares with bryophytes the regression and disappearance of the lamellar strip in mature cells (Renzaglia and Duckett, 1987, 1989, 1991; Bernhard and Renzaglia, 1995). In all other pteridophytes, the LS is intact in the mature sperm cell (Duckett, 1975; Maden et al., 1997). However, LS regression in *Selaginella* occurs equally along its length in the final stages of cellular differentiation. In bryophytes, LS regression begins in the late mid-



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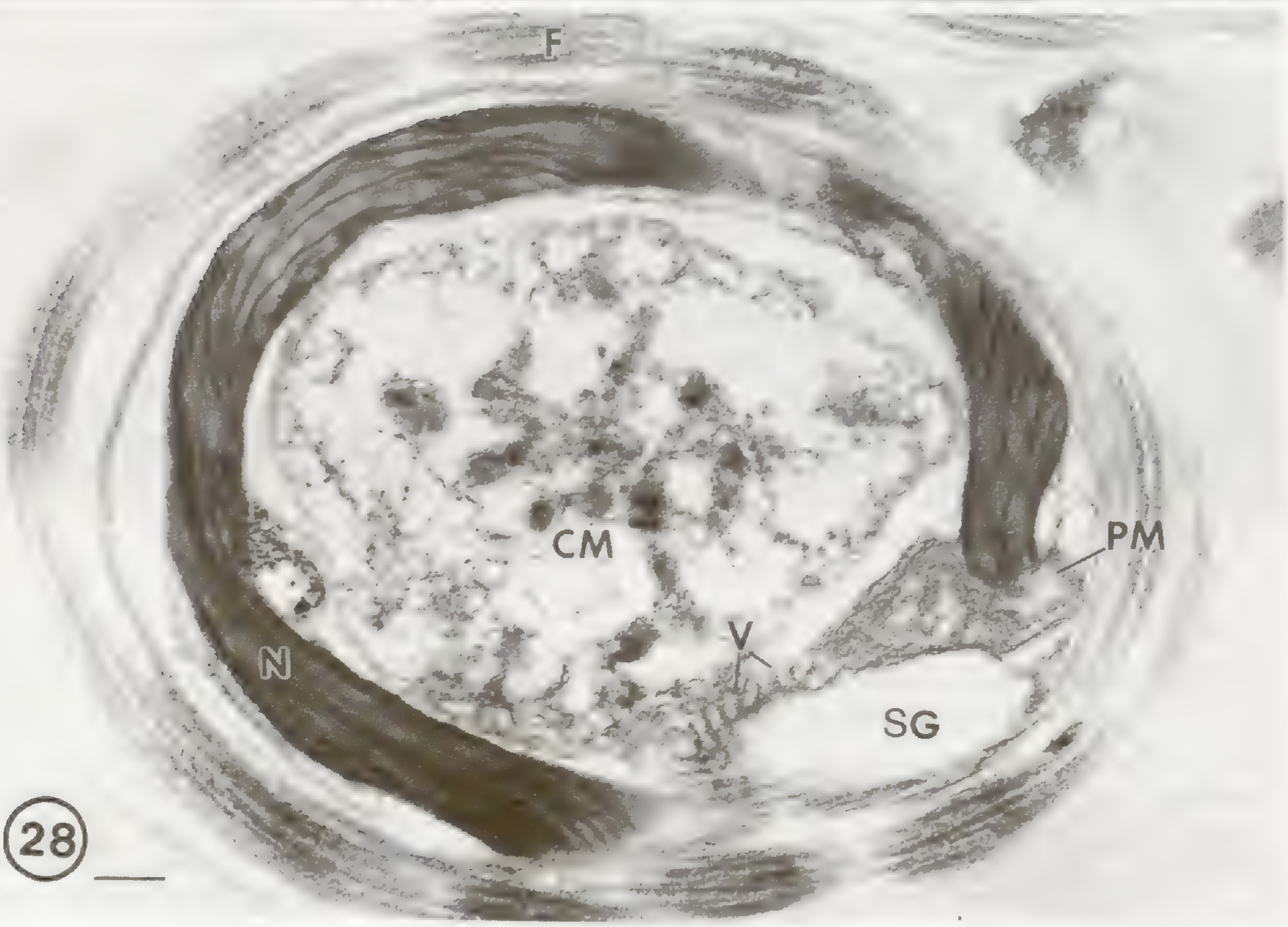

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stage and occurs gradually from posterior toward anterior, leaving a dense LS remnant at the tip of the cell (Renzaglia and Duckett, 1988).

Flagella of mosses and liverworts are staggered in insertion similar to *Selaginella,* but unlike *Selaginella,* the staggering in bryophytes is the result of extensive anterior growth of the basal bodies (Renzaglia and Duckett, 1987; Bernhard and Renzaglia, 1995). The fully developed locomotory apparatus contains elongated dimorphic basal bodies that differ markedly from the monomorphic basal bodies of *Selaginella.* Staggered monomorphic basal bodies are characteristic of lycopsids (Maden et al., 1997), *Equisetum* (Duckett and Bell, 1977), and ferns (Duckett, 1975).

The spline of bryophytes is narrow as in *Selaginella,* but the shape is reversed with the widest spline at the front of the cell and a progressive decrease in MTs toward the posterior. *Selaginella* resembles other pteridophytes in that the spline gradually increases in width posteriorly (Duckett, 1973; Duckett and Bell, 1977). Furthermore, in *Selaginella,* the spline in cross section wraps laterally around the organelles, especially the anterior mitochondrion and nucleus. This condition does not occur in bryophytes but is evident in spermatozoids of other pteridophytes and *Chara* (Duncan et al., 1997). In liverworts, such as *Haplomitrium* and *Pellia,* the wide spline is positioned at a tangent to the nucleus and barely comes in contact with the organelles (Oakley and Renzaglia, 1994; Duckett et al., 1983).

Striking parallels in spermatozoid architecture are evident between *Selaginella* and Charales (Turner, 1968; Pickett-Heaps, 1968; Duncan et al., 1997). In addition to similar arrangement and relative lengths of organelles, specific features in common between *Selaginella* and the Charales include the retention of axonemes within the cell body and the spline shape. In *Chara vulgaris* L. (Duncan et al., 1997), the spline increases in width from front to back, but it reaches a maximum of 38 MTs, approximately twice as wide as the 19 MT spline in *Selaginella.* The MTs wrap around the organelles in cross section in both organisms. Similar to *Selaginella,* the MT triplets in the basal bodies of *Chara* are impregnated with a dense matrix and the stellate pattern is faint and dense in alternating regions.

In contrast to *Selaginella* spermatozoids, those of *Chara* contain numerous undifferentiated mitochondria at the cell anterior. These organelles occupy ½ gyre at the cell anterior, compared to the AM of *Selaginella* which extends 1½ gyres around the cell. In both taxa, mitochondria do not extend to the anterior

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FIGS. 27, 28. Longitudinal sections of spermatozoids. 27) section from extreme anterior tip of cell (arrow) with anterior basal body, through nucleus in the mid-region (N_1), up to back of nucleus (N_2), PM and first starch grain in plastid at cell posterior (arrowhead); vesicles surround inner plastid surface; cytoplasmic mass is almost entirely detached and occupies interior of cellular coils; to the right, an adjacent cell shows cross section of plastid overlain by spline of 19 widely-spaced microtubules. 28) section through cellular mid-region showing nucleus with twisted rods and relationship between posterior mitochondrion and starch-containing plastid; vesicles surround inner plastid surface; cytoplasmic mass is almost entirely detached and occupies interior of cellular coils. Scale bars=0.3 μm. For labels, see legend to Fig. 1.

tip of the cell. This is in contrast to bryophyte and many other pteridophyte sperm cells (Renzaglia and Duckett, 1991; Maden et al., 1997). The multiplastidic nature of charalean sperm also is in sharp contrast to the monoplastidic sperm of *Selaginella,* some Lycopodiaceae (Renzaglia et al. 1994), and bryophytes (Renzaglia and Duckett, 1988).

It is unlikely that the variety of structural features that *Selaginella* shares with green algae is at all indicative of a common origin. Indeed, features such as the shape of the spline and occlusion of basal bodies, conditions shared by other land plants, may simply be examples of parallel evolution. Other characteristics, such as the maintenance of the flagellar axoneme within the cell, are exclusive to *Selaginella* and green algae, and as such may represent symplesiomorphies.

Elimination of the bulk of the cytoplasm is an essential component of the streamlining process. In this regard, *Selaginella* more closely resembles other pteridophytes than bryophytes or *Chara.* In *Selaginella* and most pteridophytes, the cytoplasmic deletion process is rapid, occurs in the final stages of development and involves spline microtubules (Myles and Hepler, 1977; Duckett, 1975). A large mass of cytoplasm is shed in bulk when sperm are released from the antheridium and become motile. The process is gradual in bryophytes and appears to be intimately linked with autolysis of organelles and progressive sloughing off of the cytoplasm (Miller and Duckett, 1986).

An additional feature *Selaginella* shares with pteridophytes is the location of the stellate pattern of the transition zone inside the main body of the cell instead of in the flagellar shaft. In bryophytes, the transition zone is exclusively positioned in the flagellar shaft (Pass and Renzaglia, 1995).

For an exact determination of character state homology, developmental information is critical. In view of the complexity of cellular morphogenesis, the details of spermatogenesis in *S. australiensis* will be outlined elsewhere (Renzaglia, Bernhard, and Garbary, in prep.). Unlike previous phylogenetic analyses based solely on spermatogenesis in which *Selaginella* aligns with the bryophyte and not the pteridophyte lineage (Garbary et al., 1993; Mishler et al., 1994), analyses incorporating the entire developmental data set firmly place *Selaginella* as a member of the pteridophyte line, with nesting among the lycophytes (Maden et al., 1997).

With examination of more organisms, it has become increasingly apparent that sperm cell structure and development provide a strong phylogenetic signal. The small and relatively simple spermatozoids of *Selaginella* have retained a number of features that are best interpreted as plesiomorphic among land plants. These include organellar position and number, with two mitochondria and a single plastid, and the biflagellated condition. It is crucial at this time to examine spermatogenesis in an isophyllous species of *Selaginella.* Isophyllous taxa are less diverse and exhibit less specialized features than anisophyllous forms, such as *S. australiensis* and *S. kraussiana* (Jermy, 1986; Valdespino, 1993). Comparative data on sperm structure and ontogeny in *Isoetes* also will provide significant phylogenetic information in identifying the precise placement of heterosporous lycophytes in the vascular plant lineage.

Undoubtedly, an increase in taxon sampling coupled with comprehensive information on the complex process of spermatogenesis will aid in resolving interrelationships among the diverse and ancient lycophytes.

ACKNOWLEDGMENTS

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SEM Studies on Vessels in Ferns. 5. *Woodsia scopulina*

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ABSTRACT.—Scanning electron microscopy (SEM) studies of macerations reveal that vessels are present in both roots and rhizomes of *Woodsia scopulina*. In rhizomes, there is less differentiation between lateral walls and end walls. In both root and rhizome vessels, there are perforation plates both on end walls and on lateral walls; because of the grouping of tracheary elements, more than one cell facet at a cell tip can qualify as an end wall. Lateral wall perforation plates are less specialized than end wall perforation plates. Lateral wall perforations often have porose pit membranes, whereas end wall perforations often lack pit membrane remnants, especially in roots. Tracheids may be present in addition to vessels, but because one cannot see all facets of a cell and many cells are broken or overlain, absence of perforation plates on all tracheary elements cannot be established with certainty. *Woodsia obtusa* has less specialized vessels: end wall perforation plates have porose pit membranes, and lateral perforation plates are apparently lacking. Greater specialization of vessels characterizes adaptation to more seasonal habitats in angiosperms; this correlation appears to apply to ferns as well. *Woodsia scopulina* tends to occupy sites of higher latitude or altitude than those of *W. obtusa*.

Vessels have been reported for ferns in the case of *Pteridium* (Russow, 1873; Bliss, 1939; Carlquist and Schneider, 1997a), and these reports have been widely accepted. Vessels were described in roots of *Marsilea* by White (1961, 1962), and the vessels he illustrates have clearly defined perforation plates. Vessels were claimed by Gwynne-Vaughan (1908) for several genera of ferns, but that report was discounted by Bancroft (1911). White (1962) suggested there might be vessels in ferns other than *Pteridium* and *Marsilea*, but he was appropriately cautious, because demonstration of presence or absence of pit membranes in end walls of tracheary elements by means of light microscopy, the tool available to him, is difficult or impossible. However, White (1962) singled out *Woodsia* (Dryopteridaceae) as a genus likely to have vessels (he designated them as "presumptive vessels") in roots, although not in rhizomes. White cited eight *Woodsia* species has having slight differentiation of end walls (as compared to lateral walls) on tracheary elements, two as having medium differentiation, and one as having a high degree of differentiation. One of the two species cited with medium differentiation of the end walls was *W. scopulina* D.C. Eaton.

Because SEM is a definitive tool for showing presence or absence of membranes in pits on end walls and lateral walls of fern tracheary elements, we have elected to study with SEM the ferns most likely to have vessels based on differentiation between end walls and side walls as seen with light microscopy. In *Pteridium* (Carlquist and Schneider, 1997a), *Woodsia ilvensis* (L.) R. Br. (Carlquist and Schneider, in press), *Microgramma nitida* (J. Sm.) A.R. Sm. (Schneider and Carlquist, submitted), and *Phlebodium* and *Polystichum*

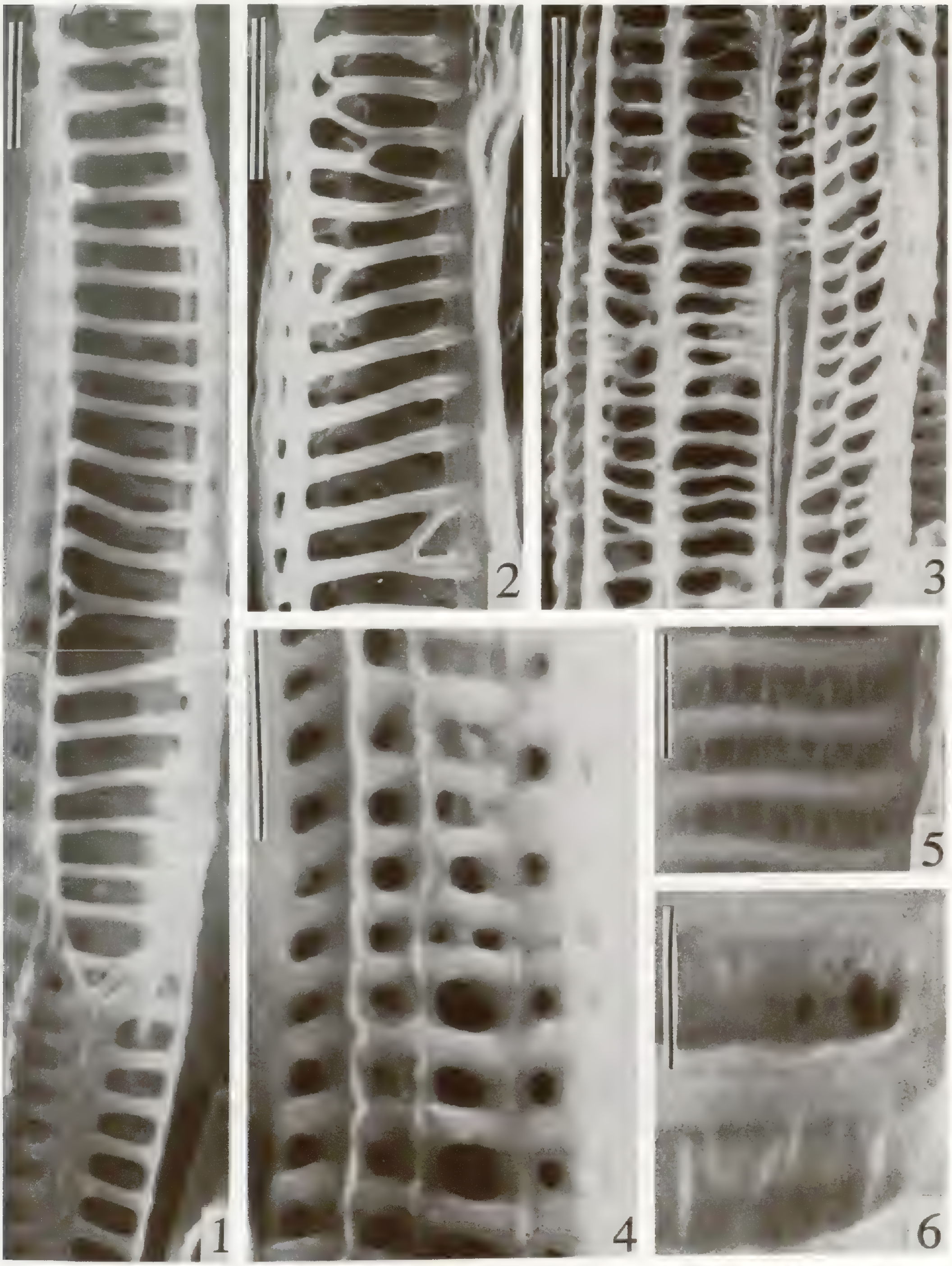
(Schneider and Carlquist, 1997), we have established the presence of well-differentiated end wall perforation plates. We have also found lateral-wall perforation plates in these genera, although determination of what is an end wall and what is a lateral wall is not as easy in ferns as it is in angiosperms, where vessels are mostly in clear vertical series and do not form dense groupings, as they do in these ferns. In *Woodsia obtusa* (Spreng.) Torr. (Carlquist et al., 1997), we found end walls on tracheary elements with porose lysis of pit membranes, and therefore we claimed perforation plates as present; we did not see perforation plates on lateral walls in this species. *Woodsia scopulina* is of potential interest because the degree of differentiation of end walls of tracheary elements, according to the data of White (1962), is greater than in *W. obtusa*. If greater differentiation between end walls and lateral walls is present in *W. scopulina,* other tracheary element features that differ from those of *W. obtusa* are potentially of interest. In our study of *W. obtusa* (Carlquist et al., 1997), we were unable to determine the full extent of perforation plates because sections were used. Use of macerations in the present study permits better understanding of the three-dimensional nature of vessel elements.

The fact that a number of ferns now prove to have vessels makes each genus and species in which vessels occur of potential interest. White's (1962) suggestion that vessels might be absent in rhizomes of *Woodsia* proves not to be true for at least some species. However, this finding leads to the question of whether rhizome vessels may be less specialized and more tracheidlike than root vessels. Also of potential interest is whether tracheids are present in addition to vessels in *Woodsia.* Differences among species with respect to vessel specialization of vessels are also of potential ecological interest, because more specialized perforation plates in monocotyledons and dicotyledons are associated with more highly seasonal availability of moisture (Carlquist, 1975). *Woodsia scopulina* occurs in areas that are cool to cold in winter, and where freezing might be a factor in water availability; warm summer temperatures and drought may characterize at least some of the localities. Brown (1964) documented the range of *W. scopulina* from southern Alaska down the Rocky Mountains to southern California and Arizona, as well as along the northern part of the Great Plains: South Dakota, northeastern Minnesota, northern Saskatchewan, Ontario, and the Gaspé area of Québec. He cited isolated stations in Arkansas and the Appalachian Mountain portions of North Carolina, Tennessee, Virginia, and West Virginia.

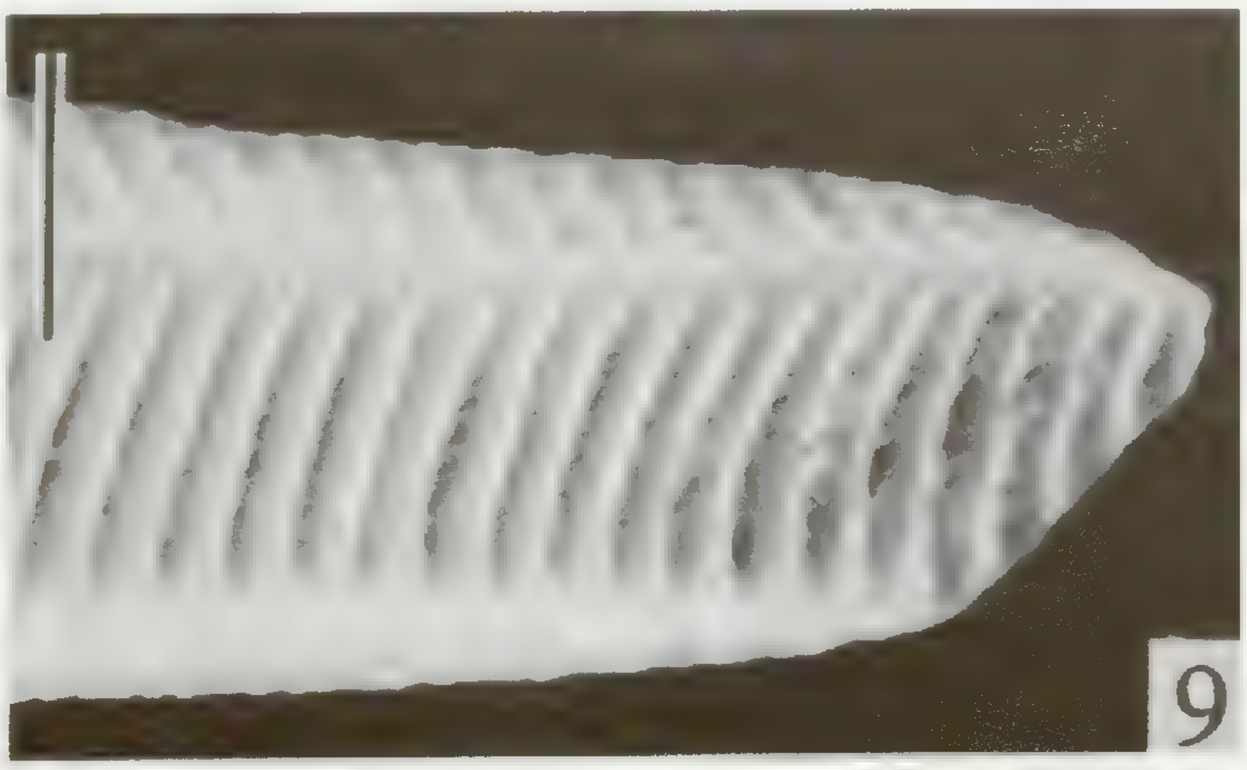
Materials and Methods

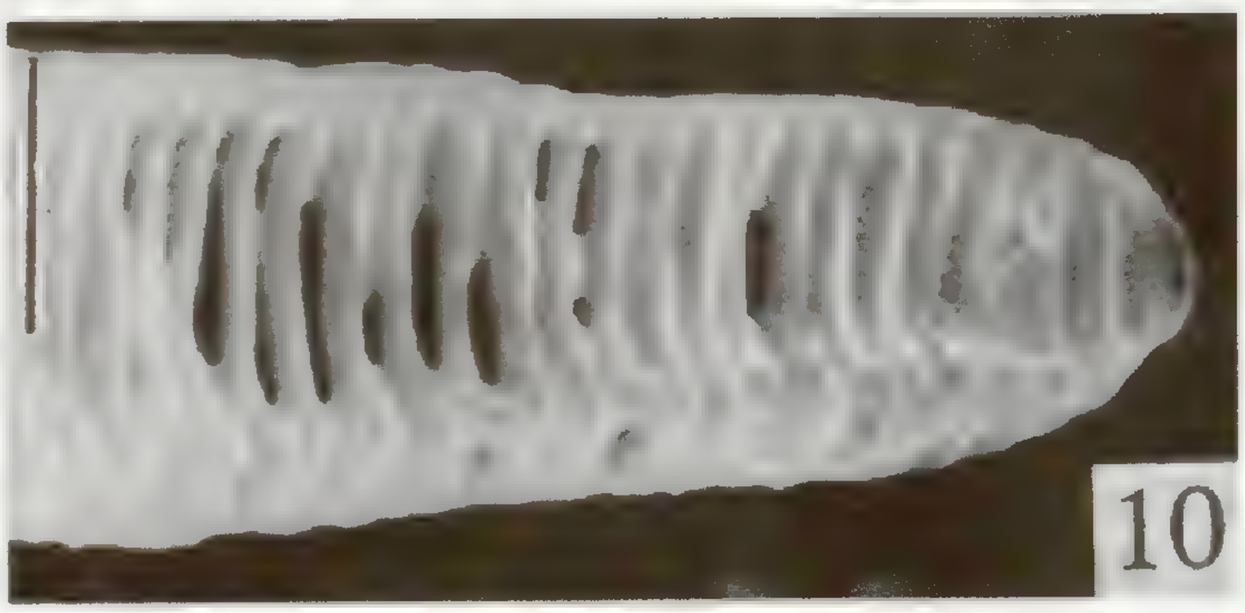
The plants of *W. scopulina* used in this study were collected in the only known locality in Arkansas, Magazine Mountain in Logan County, during October, 1996. The collectors were Don Crank, Jim Peck, and Robbin Moran. Material was sent to us in fresh condition by George Yatskievych. Segments of roots, rhizomes, and petioles of several plants were preserved in 50% aqueous ethyl alcohol.

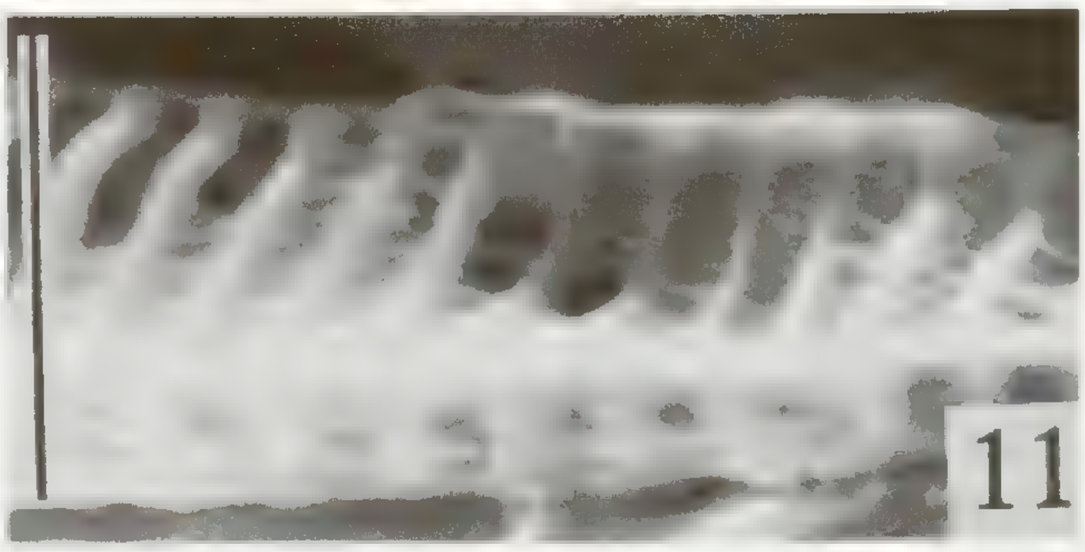
Although in our study of *Woodsia obtusa* (Carlquist et al., 1997) we sectioned



FIGS. 1–6. SEM photographs of root tracheary elements of *Woodsia scopulina*. 1) About half of end wall perforation plate with narrow bars, wide perforations; lateral wall perforations at lower right. 2) Portion of end wall perforation plate with wider bars. 3) Facets of two adjacent tracheary elements, showing perforations on all faces except one shown obliquely at extreme right. 4) Four adjacent facets of a single tracheary element, at least some of which represent lateral walls; all walls bear perforations. 5) Lateral wall of tracheary element; striate pit membranes are present. 6) Portions of two lateral wall pits; in upper pit membrane, small pores are present at right. Scale bars: Figs. 1–4, bars at upper left=10 μm; Fig. 5, bar=5 μm; Fig. 6, bar=2 μm.







FIGS. 7–13. SEM photographs of rhizome tracheary elements of *Woodsia scopulina*. 7) Walls of tracheary elements bearing pits with intact striate pit membranes. 8) Central portion of surface of tracheary element; left two-thirds of photograph is a perforation plate (likely an end wall) with wide bars, narrow perforations; porose perforations occur in the pits of cell facet at right. 9–12. Tips of tracheary elements to show perforations. 9) Perforation plate with a few pores in pit membranes near cell tip. 10) Elongate pores are present in about half of the pits of the perforation plate. 11) Circular to elongate pores are present in about half of the pits; pit membranes are striate.

xylem, excessive fracturing has since led us to prefer maceration. Material of *W. scopulina* was macerated in Jeffrey's Fluid and storied in 50% aqueous ethanol. Macerated portions were dispersed over the surface of adhesive conductive circles attached to aluminum stubs, dried, sputter-coated, and examined with a Bausch and Lomb Nanolab SEM. In our SEM study of *Pteridium* tracheary elements (Carlquist and Schneider, 1997a), we used both sectioned and macerated material. On the basis of comparing materials prepared according to those respective methods, we have observed that pit membranes are not altered by our maceration methods and represent essentially the same characteristics as those seen in material that has been embedded in paraffin and sectioned.

Results

In roots of *W. scopulina,* long perforation plates with numerous narrow bars may be seen on end walls (Fig. 1). The spaces between the bars are wide and devoid of pit membrane materials in the end wall perforation plate shown in Fig. 1. In the end wall perforation plate shown in Fig. 2, bars are slightly wider, separated by somewhat narrower spaces, and pit membrane remnants are also absent. Forking and anastomosis of bars of the perforation plate may be seen in Fig. 2.

Lateral wall perforation plates also occur in the roots of *W. scopulina*. The fact that a perforation plate may be seen on the wall facet, lower right in Fig. 1, below an end wall perforation plate shows that an end wall perforation plate can co-occur with a lateral wall perforation plate. Similarly, portions of perforation plates side by side can be seen on vessel elements in Figs. 3, 4. Small areas of pit membrane remnants are visible in these perforation plate areas (Fig. 3, center; Fig. 4, upper right). The fact that several perforation plates can occur on the facets of a tracheary element indicates that a single end wall perforation plate cannot be identified. Although a tracheary element can be said to have more than one end wall where the tip contacts portions of several adjacent tracheary elements, we believe that at least some facets illustrated bearing perforations should be designated as lateral walls. Some lateral wall facets bear pit membranes, some with striations in our preparations (Fig. 5). At greater magnification, formation of small pores is evident (Fig. 6); this area may represent a lateral wall with very little differentiation into a perforation plate.

Rhizome tracheary elements are shown in Figs. 7–13. In Figs. 7, 8, facets we believe to represent lateral walls are shown. The wall surfaces of Fig. 7 bear pits covered with pit membranes; the pit membranes are notably striate. The cell facet at left in Fig. 8 may be a portion of a very long end wall; at any rate, it is clearly part of a perforation plate. The perforation plate at left in Fig. 8 lacks pit membrane remnants; bars are relatively wide and perforations rela-

←

12) Membranes are present in five pits at cell tip; perforations to the left of those pits. 13) Two rows of perforations at cell tip; strands of cell wall material cross some of the perforations. Scale bars: **Figs. 7–13, bars at upper left=10 μm.**

tively narrow. The cell facet at right in Fig. 8 is a perforation plate with relatively little differentiation; some pores are present in some pit membranes, other pits lack pores, but all of the pit membranes show striation. The series of tips of tracheary elements shown in Figs. 9–13 illustrates degrees of differentiation of end wall as a perforation plate. The end wall shown in Fig. 9 shows pores in only a few of the pit membranes. In Fig. 10, pores or holes of various size are present in more than half of the pit membranes of the end wall. In Fig. 11, striate pit membranes are shown, and pores of various sizes are present in about half of the pits. A narrow tracheary element is shown in Fig. 12; the five pits closest to the tip are not perforated, but those to the left of those pits are all perforated. In Fig. 13, two end wall facets of a tracheary element are illustrated; most of the pits in these end walls have perforated membranes, and strands of wall material can be seen in some of the perforations.

In general, the perforation plates of tracheary elements in the rhizomes are less well differentiated from lateral walls than are the perforation plates in root tracheary elements. In both roots and rhizomes, we observed some tracheary elements on which we could not see any perforations. Perforations may have been present on faces of those cells which were not visible, however. On this account, we cannot definitely say that tracheids are present or how abundant they are. Breakages in cells and overlying cellular material in a preparation also prevent one from obtaining information about the entirety of any particular cell. Taking these factors into account, we believe that at most, tracheids are less common than vessel elements in both the roots and rhizomes of *W. scopulina.*

Discussion and Conclusions

Vessel elements are clearly present in *W. scopulina;* some tracheids may also be present. If one compares the vessel elements of *W. scopulina* to those described for *W. obtusa* (Carlquist et al., 1997), the vessel elements of *W. scopulina* show more differentiation of perforation plates. In *W. obtusa,* perforation plates in both roots and rhizomes appeared confined to end walls; in addition, perforations were characterized by presence of porose pit membranes. In *W. scopulina,* perforation plates in both roots and rhizomes are present on both end walls and lateral walls, conceding that any given tracheary element may have several end wall facets (and thus, several end walls) as well as lateral walls. In *W. scopulina,* the end wall perforations are more clearly defined than they are in *W. obtusa*; bars are generally thinner, perforations are wider, and pit membrane remnants are fewer. In rhizomes of *W. scopulina,* some vessel elements with incipient formation of perforation plates (e.g., Fig. 9) are present, and these lead us to conclude that perforation plates in rhizomes are, in general, less specialized than those of roots ("specialization" is used here to connote appreciable difference between an end wall perforation plate and a lateral wall area bearing only pits). In both roots and rhizomes of *W. scopulina,* however, there is a range in specialization in perforation plates, as the series in Figs. 9–13 indicates).

The presence of lateral perforation plates in addition to those on end walls has

been reported in ferns in *Pteridium* (Carlquist and Schneider, 1997a), *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997), and *Astrolepis* (Carlquist and Schneider, 1997b). The fact that tracheary elements occur in ferns in strands in which any given tracheary element is typically in contact with several other tracheary elements may be correlated with this phenomenon. To date, perforation plates on lateral walls have not been reported in angiosperms; we did not observe lateral perforation plates in xylem of *Woodsia obtusa* (Carlquist et al., 1997).

The degree of specialization of perforation plates in *W. scopulina* is greater than that in *W. obtusa,* but less than that in *W. ilvensis* (Carlquist and Schneider, in press). In both dicotyledons and monocotyledons, degree of specialization of perforation plates is related to ecology, with more specialized plates in more strongly seasonal habitats (Carlquist, 1975). Do such correlations occur in ferns? The distributions of *W. obtusa* and *W. scopulina* are both extensive, but the specimen citations by Brown (1964) suggest that the localities of *W. scopulina* are mostly in somewhat higher latitudes and altitudes than those of *W. obtusa.* The localities of *W. ilvensis* are likely subject to greater seasonal extremes than those of *W. scopulina* (Carlquist and Schneider, in press).

Woodsia as a whole occurs in habitats rather extreme for ferns—often on exposed cliff faces or rocky slopes where drought and cold may be pronounced. One might expect that in *Woodsia* as a whole, perforation plates would be more specialized than in most other ferns. Such ecological comparisons among genera must await further studies, because our knowledge of vessel occurrence in ferns remains incomplete.

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An Autoradiographic Investigation of Protein Synthesis During Rhizoid Differentiation in *Pteris vittata*

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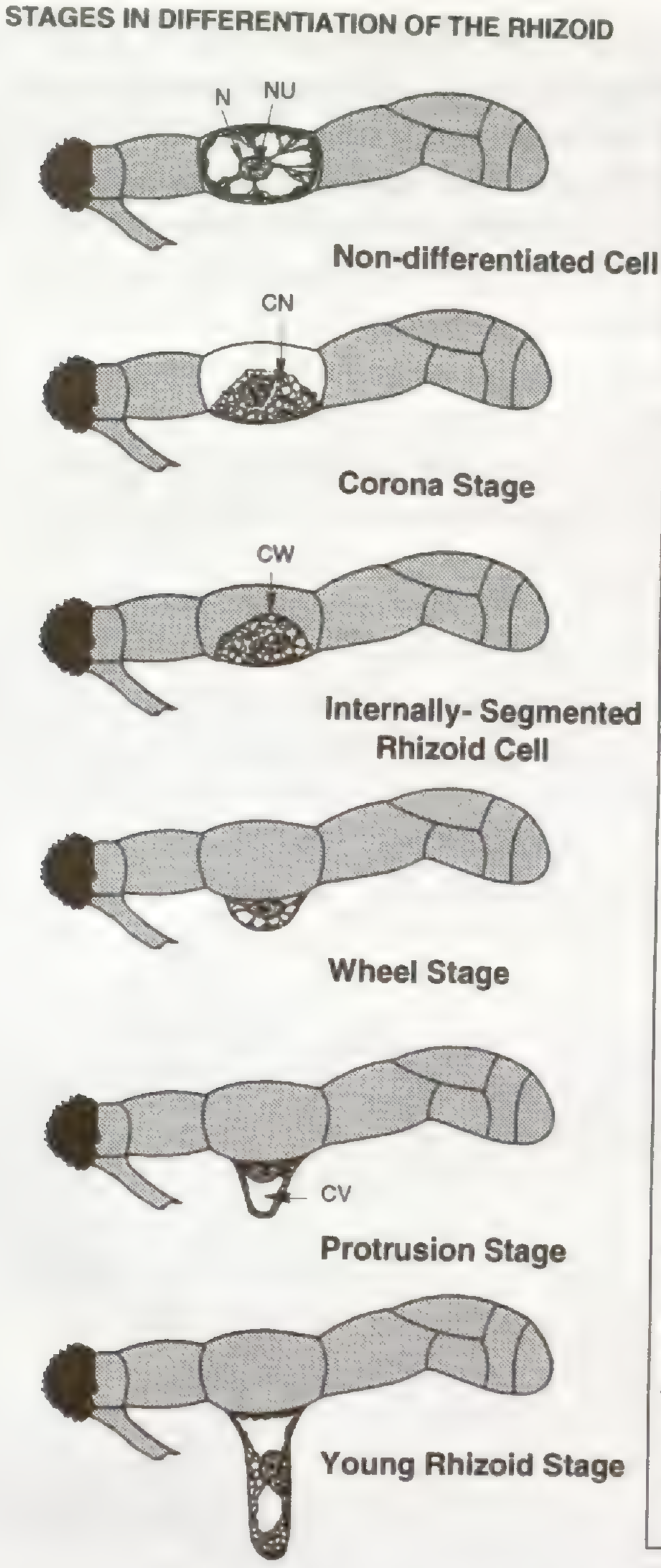
ABSTRACT.—In this autoradiographic study of protein synthesis during rhizoid differentiation in *P. vittata*, total grain counts increase seven-fold, from 42 grains in the non-differentiated stage to 295 in the premitotic corona stage. This almost doubles to 624 in the postmitotic internally-segmented rhizoid stage (irc) and increases five times to 3,079 in the wheel stage that follows; the count then doubles to 6,120 grains in the later protrusion stage and approximately doubles again to 13,487 in the final young rhizoid stage. These results show that rhizoid initiation, differentiation, and growth are attended by significant increases in protein syntheses. Although the grain count over the cytoplasm increases seven-fold in the corona when compared to the non-differentiated cell, the relative percentage distribution for this compartment remains unchanged. However, grain counts over the cytoplasm of postmitotic stages increase from 80% (irc) to 96% (wheel) to 97% (protrusion) and to 99% (young rhizoid) of the total count. In contrast, nuclear labeling reached a stationary phase in the irc and in the subsequent postmitotic stages.

The first rhizoid in *Pteris vittata* L. is produced when the spore divides mitotically at germination; however, rhizoid formation may be more clearly observed later in gametophyte development, i.e., in the enlarged, vacuolated thallus cells that mature behind the regions of cell division and enlargement (Crotty, 1967).

Increases in cytoplasmic RNA in the vicinity of the spore nucleus during primary rhizoid formation in *Matteuccia struthiopteris* (L.) Tod. were reported by Gantt and Arnott (1965). Extensive cytochemical studies of *Pteris vittata* by Crotty (1961, 1967) have provided detailed descriptions and analyses of the events at the light microscope level that accompany secondary rhizoid development, including RNA, protein, and polysaccharide syntheses. Additionally, these studies described a succession of developmental stages that occurred during the pre- and postmitotic rhizoid differentiation process (Fig. 1). Using a precise autoradiographic technique, Cohen and Crotty (1979) reported that the RNA in the anastomosing cytoplasmic strands around the nucleus of the corona (first stage of rhizoid differentiation) originated in the premitotic nucleus of this stage. In a later autoradiographic study, Cohen and Crotty (1990) demonstrated the existence of specific patterns of RNA synthesis in the stages of rhizoid differentiation in *P. vittata*.

The purpose of this study was to determine if protein synthesis during rhizoid differentiation, as measured by radioactive leucine uptake, could also be quantified and resolved into specific patterns. And, if so, can the results show a meaningful relationship to the earlier studies in *P. vittata*? In fact, the results of this investigation demonstrate that rhizoid differentiation is attended by



Compartment	Total counts 4 cells	% Total counts +/- sd
Nucleoplasm	27	64.6 +/-2.5
Nucleoli	0	0.0
Nucleus	27	64.6 +/- 2.5
Cytoplasm	15	35.4 +/- 2.5
Background	0	0.0
Total	42	
Nucleoplasm	159	53.9 +/- 0.9
Nucleoli	10	3.8 +/- 0.6
Nucleus	169	57.3 +/- 1.1
Cytoplasm	105	35.3 +/- 1.9
Background	21	7.4 +/- 2.4
Total	295	
Nucleoplasm	116	17.8 +/- 2.8
Nucleoli	5	0.6 +/- 0.7
Nucleus	121	18.4 +/- 3.5
Cytoplasm	492	80.4 +/- 4.9
Background	11	1.3 +/- 1.5
Total	624	
Nucleoplasm	110	3.5 +/- 1.3
Nucleoli	5	0.2 +/- 0.1
Nucleus	115	3.7 +/- 1.2
Cytoplasm	2950	95.9 +/- 0.9
Background	14	0.5 +/- 0.5
Total	3079	
Nucleoplasm	113	1.8 +/- 0.8
Nucleoli	5	0.1 +/- 0.1
Nucleus	118	2.0 +/- 0.8
Cytoplasm	5989	97.7 +/- 0.7
Background	13	0.2 +/- 0.1
Total	6120	
Nucleoplasm	127	1.0 +/- 0.2
Nucleoli	10	0.1 +/- 0.1
Nucleus	137	1.0 +/- 0.2
Cytoplasm	13329	98.8 +/- 0.2
Background	21	0.2 +/- 0.1
Total	13487	

FIG. 1. Sequential stages of rhizoid differentiation in *P. vittata* with absolute grain counts per compartment (nucleus, nucleolus, cytoplasm and background) expressed as relative percentages (with standard deviations, SD) of the sum total of grain counts from four similar cells for each stage. Abbreviations: N=nucleus, NU=nucleolus, CN=corona network, CW=cell wall, CV=cell vacuole.

definite patterns of protein syntheses that can be quantified and correlated with the earlier RNA studies.

MATERIALS AND METHODS

Many of the procedures used in this study were detailed in earlier publications by Crotty (1967), Cohen et al., (1973), and Cohen and Crotty (1979,

1990). Spores of *P. vittata* were washed and placed into sterile Falcon tissue culture flasks, containing 4 ml of a liquid growth medium modified after Shive and Robins (1937). During germination and growth, gametophytes were incubated at 25 ± 2°C under a bank of two fluorescent (20 watts each) and two incandescent (40 watts each) lamps, which provided a light intensity of 25 foot candles (ft-c) at a distance of 40 cm from the surface of the flasks. After ten days of growth, the nutrient medium was replaced with fresh medium, and the gametophytes were carried through the successive processes of incubation in radioactive precursor, fixation, and dehydration.

Growth was continued for 20 days after sowing, before rhizoids were induced by the following procedure. Gametophytes were first placed in the dark for 29 hr and then exposed to a stimulating light intensity of 40 ft-c by raising the flasks 15 cm above the position they occupied during initial germination and growth. With this procedure, the several stages of rhizoid differentiation were harvested for fixation following specific exposures to light stimulation. For example, corona stages were found after 18.5 hr of light stimulation; internally-segmented rhizoid cells (irc) after 19.5 hr; wheel stages after 21.5 hr; protrusion stages after 23.5 hr; and young rhizoid stages after 25.5 hr. Nondifferentiated thallus cells that had not demonstrated any cytological evidence of rhizoid cell differentiation were selected from gametophytes fixed at the same time as the corona stage, i.e., after 18.5 hr of light stimulation.

H^3-leucine (New England Nuclear Co.) was diluted in fresh liquid nutrient medium to a final concentration of 2.0 μC/ml. The old medium was poured off, and 4 ml of the radioactive nutrient medium was added to the tissue culture flasks for the final 30 min of each stimulating photoperiod. After isotope incorporation, gametophytes were rinsed with nonradioactive medium, fixed in 3% glutaraldehyde in Millonig phosphate buffer (Millonig, 1961), and dehydrated through a graded series of ethyl alcohols (EtOH). The gametophytes were then dehydrated in 100% EtOH and infiltrated through a graded series of low-viscosity epoxy resin and 100% EtOH (Spur, 1969) prior to being embedded in pure epoxy.

Specimens were selected and mounted on blocks, after which serial sections 1 μm thick were cut with a diamond knife on a Reichert OM U2 ultramicrotome and mounted on glass slides. All sections of each cell had to be present for the cell to be processed for quantitative analysis. These slides were dipped in Kodak NTB-2 emulsion (Rochester, NY) diluted 1:1 with glass-distilled water (Rogers, 1969) and developed after a 20-day exposure. The slides were observed microscopically using a phase-contrast oil immersion lens. Grains were counted in serial sections of four similar cells for each of the stages over the following cellular compartments: (a) nucleoplasms, (b) nucleoli, (c) nuclei, (d) cytoplasms, and (e) backgrounds. Both absolute grain counts and percentage values per cellular compartment per stage were reported in this study. However, for statistical purposes, only the percentage values and their associated standard deviations were compared. The Student's t-test for small samples (Bancroft, 1957) was used to evaluate the probabilities of statistical sig-

nificance. Only differences with t-values significant beyond the 0.5 probability level were reported as significant in this study.

Autoradiographic controls performed for background levels, e.g., for the generation of false latent images and the fading of latent images (Rogers, 1969), showed that neither false positives nor false negatives were a problem in this study. Control experiments using 2.0 mg/ml crystalline pepsin (Spiro, 1978) verified the specificity of H^3-leucine uptake into proteins synthesized in the stages of rhizoid differentiation with a 90% reduction of grain counts. Stage comparisons were done according to the procedure of Cohen and Crotty (1990).

Results

Total Overall Grain Count Changes per Stage During Rhizoid Differentiation.—When total grain counts were compared during rhizoid development, a seven-fold increase, from 42 grains (non-differentiated) to 295 grains (corona) was observed. In the irc stage, the total count of 624 was double that of the earlier corona stage. This increase continued as development progressed. For example, the wheel stage count of 3,079 grains was five times that of the earlier irc stage; protrusion stage with 6,120 grains was double that of the previous wheel stage; and young rhizoid stage with 13,487 grains had a two-fold increase over protrusion stage. Comparisons of the total grain counts between the young rhizoid stage and each of the earlier stages demonstrated that the young rhizoid stage had a total count 321 times larger than the non-differentiated stage, 45.7 times that of the corona stage, 21.6 times that of the irc stage, 4.4 times that of the wheel stage, and 2.2 times that of the protrusion stage.

Analysis of Compartment Labeling (Absolute Grain Counts and Percentages) Within Each Stage and Between Successive Stages.—Non-differentiated and Corona Stages.—In the nucleoplasm, the grain count increased approximately six-fold, from 27 in the non-differentiated to 159 in the corona, while in the nucleolar compartment, the count went from zero to ten, respectively. The cytoplasmic counts increased approximately seven-fold, from 15 in the non-differentiated stage to 105 in the corona. Relative percentages for these two stages showed that there was a proportionately equal increase in grain count over the cytoplasm which remained statistically unchanged, at 35%. Although the nuclear percentage appeared to decrease significantly, i.e., from $64.6 \pm 2.5\%$ in the non-differentiated stage to $57.3 \pm 1.1\%$ in the corona (Fig. 1), one should be cautious in interpreting its biological meaning, given the presence of background grains over the corona (approximately 7%), the absence of background grains over the non-differentiated stage, and the relatively low counts involved. When compared to all subsequent stages, both the non-differentiated and corona had a higher percentage of radioactive label in the nucleus than in the cytoplasm (Fig. 1).

Internally-segmented Rhizoid Cell.—In the irc, there was a shift in the pattern of H^3-leucine uptake when compared to the corona. Labeling in the irc was greater over the cytoplasmic compartment. Specifically, there were 121

grains over the nucleus and 492 grains over the cytoplasm, as opposed to 169 over the nucleus and 105 over the cytoplasm of the corona. Percentage values showed the incorporation shift from nucleus to cytoplasm was even more apparent and statistically significant. For example, in the corona 57.3% of the grains were over the nucleus and 35.3% over the cytoplasm, whereas in the irc stage 18.4% were over the nucleus and 80.4% were over the cytoplasm (Fig. 1). This dramatic shift can be mainly accounted for by the increase of cytoplasmic counts rather than by the decrease in nuclear counts. For example, cytoplasmic counts increased five times, from 105 in the corona to 492 in the irc, whereas the nuclear counts decreased by a factor of about 0.3, going from 169 in the corona to 121 in the irc.

WHEEL STAGE.—The trend of increased cytoplasmic uptake of H^3-leucine continued in the wheel stage (and in subsequent stages). Between the irc and wheel stages, there was very little change in counts in the nuclear compartments, e.g., 121 (irc) versus 115 (wheel). However, cytoplasmic counts increased six-fold, from 492 (irc) to 2,950 (wheel). It was this change in cytoplasmic counts that accounted for the continued shift in percentage distribution between the nucleus and cytoplasm. That is, the 18.4 ± 3.5% (nuclear) and 80.4 ± 4.9% (cytoplasmic) distribution in the irc shifted to 3.7 ± 1.2% (nuclear) and 95.9 ± 0.9% (cytoplasmic) in the wheel stage (Fig. 1).

PROTRUSION AND YOUNG RHIZOID STAGES.—The same shift in pattern discussed in the wheel stage continued in protrusion and young rhizoid stages. For example, nuclear counts remained at the same level as seen in the irc and wheel stages. The cytoplasmic count in the protrusion stage increased two-fold, from 2,950 (wheel) to 5,989 (protrusion); similarly, the cytoplasmic count in the young rhizoid stage doubled again to 13,329. The percentage distribution reflected this massive cytoplasmic increase in grain counts so that the nuclear and cytoplasmic percentages were 2.0 ± 0.8% and 97.7 ± 0.7%, respectively, for the protrusion stage and 1.0 ± 0.2% (nucleus) and 98.8 ± 0.2% (cytoplasm) for the young rhizoid stage (Fig. 1).

MAJOR PATTERNS AND TRENDS OBSERVED IN H^3-LEUCINE UPTAKE.—The first and foremost trend was the rapid increase in counts as cell differentiation and growth proceeded from the non-differentiated cell to the young rhizoid. Total counts increased from 42 in the non-differentiated cell to 13,487 in the young rhizoid, an increase of approximately 321 times. The rate of increase was not uniform however, but varied with stage and compartment. For example, a seven-fold increase occurred between non-differentiated and corona, then approximately doubled between corona and irc stage. From the irc to wheel stages, there was a five-fold increase. Thereafter, it doubled between the wheel and protrusion stages and doubled again between the protrusion and young rhizoid.

A second major pattern was observed in the corona stage, which, like the non-differentiated stage, showed a higher percentage of label in the nuclear compartment than in the cytoplasm. However, in the case of the corona, the

nuclear and cytoplasmic compartment counts had increased six- and seven-fold, respectively, thus emphasizing the relative importance of newly synthesized proteins for nuclear associated functions in the premitotic corona stage.

A new or reversed pattern is apparent in the irc stage, where the five-fold increase in label over the cytoplasm is accompanied by a slight reduction of nuclear labeling. This is in sharp contrast with the labeling pattern of the corona stage.

Another clear pattern occurred with regard to the nuclear labeling. For example, there were essentially no changes in the counts over the nuclear compartment starting with the irc and continuing through the young rhizoid stage. In contrast, the cytoplasmic labeling continued to increase. An approximate five-fold increase between the corona and the irc was followed by a six-fold increase between the irc and the wheel; then a doubling between the wheel and the protrusion; and approximately another doubling between the protrusion and the young rhizoid. These dramatic increases in cytoplasmic counts combined with the static nuclear counts resulted in the observed shift towards the relatively high percentage of cytoplasmic labeling seen in this study.

Discussion

PATTERNS OF PROTEIN SYNTHESIS DURING RHIZOID CELL DIFFERENTIATION.—It has been shown that proteins can be synthesized in the cytoplasm and move rapidly (80 sec) into the nucleus (Wu and Warner, 1971). Also, proteins have been described as moving from the nucleus to the cytoplasm and back to the nucleus (Goldstein, 1974). Therefore, the presence of H^3-leucine may be the result of complex interactions between different cellular compartments. However, the general conclusion of Goldstein (1970) that protein is synthesized first in the cytoplasm then moves to other compartments was adopted for this study.

In *P. vittata*, rhizoid differentiation is started by the triggering of a parent thallus cell (non-differentiated stage) to divide and produce two different daughter cells, with only one, the irc, embarking on differentiation and growth processes. Several different roles can be suggested for newly synthesized proteins in the nucleus of the non-differentiated cell, which is preparing for division. For example, enzymes to trigger DNA synthesis; histones; enzymes for RNA replication; and proteins associated with new and increased RNA synthesis as indicated by Cohen and Crotty (1990).

In the case of the corona stage, tentatively assigned to early prophase, one or more of the above processes may be continuing. Other possibilities are proteins associated with nuclear ribosomal processing; precursor proteins for spindle moving from the cytoplasm into the nucleus, which most likely, accounts for a large part of the dominant nuclear labeling in the corona stage; and precursor proteins for phragmoplast and new cell wall, in anticipation of the impending cell division.

The striking postmitotic increase in grain counts over the cytoplasm, coupled with the static counts over the nuclear compartment, first becomes apparent in the irc stage. It is consistent with earlier suggestions that the irc is

the, "assembly of the growth machinery" stage, where the enzymes necessary for differentiation and growth are produced (Crotty, 1967; Crotty and Cohen, 1990). Cell wall softening enzymes have to be made, along with synthesis of many different wall components, some of which might be unique to the rhizoid cell. Others, however, may be common to both rhizoid and thallus cells, but presumably most have to be resynthesized since the parent cell wall was not growing. All of these required activities are supported by the observation that labeled protein in the irc cytoplasm has risen approximately five-fold over that of the corona. This is consistent with the observation of a two-fold increase of labeled RNA from corona to irc as indicated by Cohen and Crotty (1990).

Crotty (1967) suggested that the wheel stage represented a new synthetic activity closely associated with the beginning of the outgrowth of the rhizoid. This study not only confirms cytochemical observations that a heavy synthesis of protein marks the wheel stage, but also demonstrates that protein synthesis increases to a level that is six times greater than in the irc cytoplasm. The fact that counts in the nuclear compartments remain at a steady level from irc to young rhizoid suggests that this level of newly formed protein present in the nucleus is necessary first to create and then to maintain the rhizoid growth machinery, which has actually reached a plateau back at the irc stage.

In the protrusion and young rhizoid stages, the trend towards an increase in H^3-leucine uptake, i.e., protein synthesis, continues. This means that the nuclear contribution to the ribosomal machinery must be continuing at an adequate level, although constant. However, at the same time, the ribosomes and other components, e.g., heterogeneous RNAs, can now be reutilized during cytoplasmic protein syntheses in the growing cell. This conclusion supports the observations of Cohen and Crotty (1990) who showed RNA labeling reached a stationary phase in the wheel stage.

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The History and Classification of the Farley Fern, *Adiantum tenerum* 'Farleyense'

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ABSTRACT.—*Adiantum tenerum* 'Farleyense' is confirmed as a cultivar, not as a species or as a variety, as it is treated by some authors. The horticultural history of the fern is summarized, beginning on a Barbados sugar plantation. A neotype for the species name and a standard for the cultivar name are selected and justified. 'Farleyense' is compared morphologically with wild and cultivated *Adiantum tenerum*, as well as other maidenhair ferns, and is found to be morphologically consistent with classification as a cultivar of *A. tenerum*.

Seldom are cultivars as prominent through time, as well known, or as troublesome taxonomically as the Barbados Farley fern. Discovered on a horticulturally sophisticated Barbados sugar plantation in the mid-nineteenth century, the Farley fern won instant acclaim in British horticultural circles, and was promptly named as a species. The fern was thought, with reservations, at the time to have been derived from *Adiantum tenerum* Sw., which is abundant wild and cultivated in Barbados. The Farley fern spread to gardens around the globe, and has remained popular ever since. Despite—or possibly on account of—its horticultural prominence, the fern's history, biology, classification, and nomenclature are befuddled throughout the literature dealing with it. The fern has been treated variously as a species, variety, and as a cultivar. The aim of the present account is to provide an historical background on the Farley fern, to correct nomenclatural problems, and to provide a systematic morphological appraisal of its classification as a cultivar of *A. tenerum*.

HISTORY

By the time the Farley fern was discovered in ca. 1865, most of Barbados (Fig. 1), then a British colony, had been dominated by sugar cultivation for more than a century. Barbados sugar grew on enormous, often-prosperous plantations surrounding splendid great houses. Most of the surface of Barbados was then, and remains, under a lawn of sugar cane. The plantation owners were often well-to-do, well traveled, and extravagant. Farley Hill was one of the last and most magnificent sugar mansions (Barbados Parks and Beaches Commission, Clarke). From the estate's breezy hilltop perch some 900 feet above sea level, postcard vistas sweep eastward across the rocky coast to the Atlantic Ocean. In its prime, Farley Hill was the most imposing mansion on the island, and its gardens were famous.

Built probably in 1818, the originally modest house was known as Grenade Hall. Joseph Lyder Briggs took possession in 1856, but never lived there. In 1856, his son, Baronet Thomas Graham Briggs (1835–1887), one of the wealth-

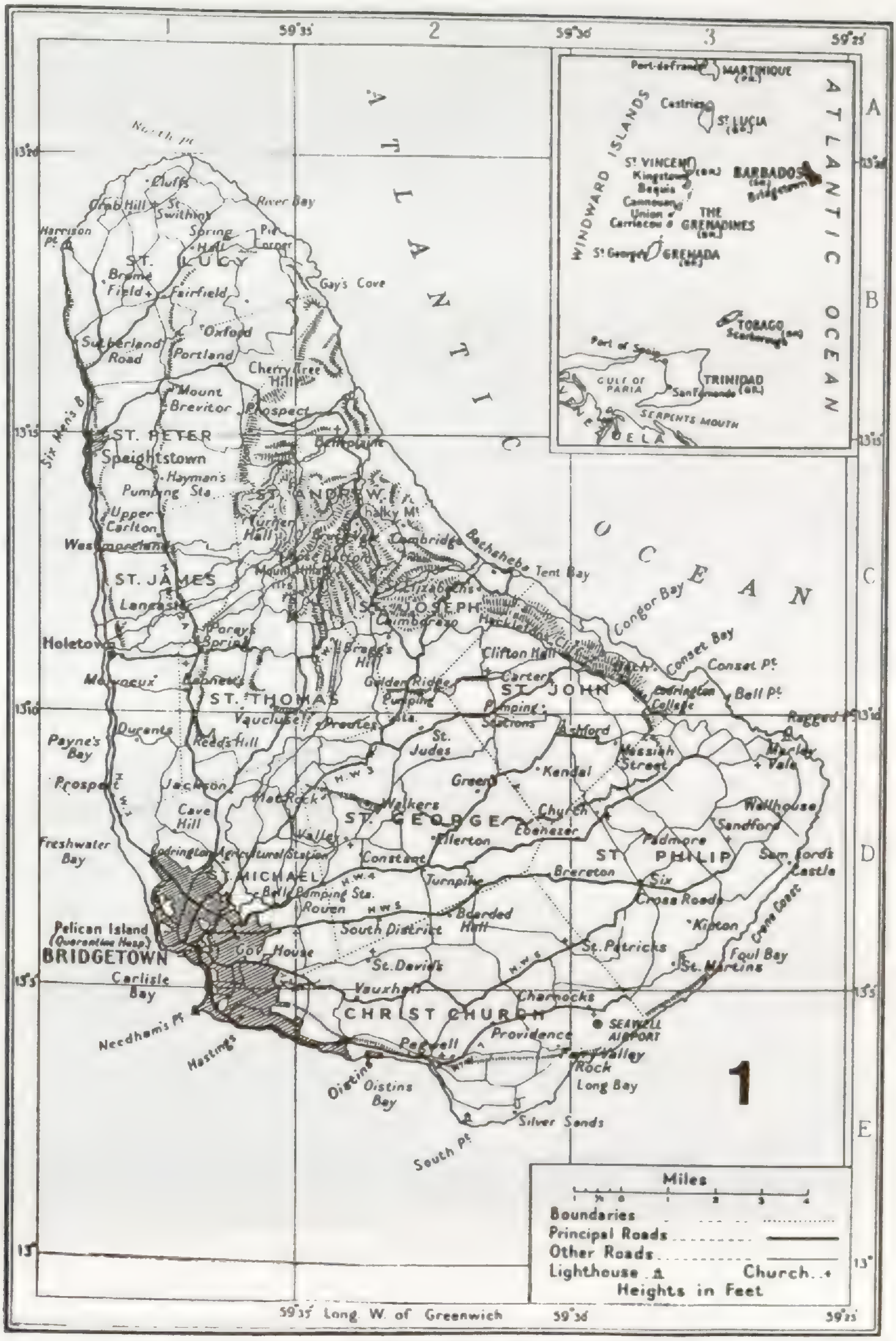

FIG. 1. Map of Barbados. The top of the map is north. Farley Hill is located at 13°15' N Lat., at the bend in the road just above Prospect.

FIG. 2. Living specimen of 'Farleyense' at Andromeda Botanic Gardens, Barbados.

iest and most cosmopolitan Barbadians of the time, took over, moved in, and renamed the property Farley Hill. From 1857–1876, Briggs developed Farley Hill into one of the finest country residences in the Caribbean. Briggs became influential in Barbados politics, retained extensive social connections off the island, and entertained vacationing monarchs.

Sir Briggs's time at Farley Hill featured horticultural enthusiasm. An observer described the gardens as "once the wonder of the island." This undoubtedly was the context for the discovery and propagation of the Farley fern (Fig. 2). Development of the property began in 1857, and the fern first appeared in the (British) literature in 1865. By the time Briggs died in 1887, his gardens had gone into decline. Hence, the fern apparently was discovered, propagated, and sent to England during the period of 1856–1865. Puzzlingly, Coulter (1968) stated with no substantiation that the Farley fern was found at Farley Hill, "in 1840 by Sir Graham Briggs, Bart., who was living there at the time." This date is apparently far too early.

After 1887, the fortunes of the house declined. Its twentieth century résumé includes serving as rental property, a movie set, and near-destruction by fire. Today, it is a hauntingly fascinating gutted ruin (Fig. 3), adding interest to what is now Farley Hill National Park. The Barbados National Conservation Commission continues the horticultural tradition of the site, maintaining beautiful public gardens featuring some of the original plantings.

The first account of the Farley fern came from Thomas Moore (1866a) of the Chelsea Botanic Garden. His exact words in an addendum to the protologue,

FIG. 3. Farley Hill today.

probably added in proof, are worth quoting. One sentence sets the record straight on matters confused by subsequent writers: "I have learned that this fern is a seedling raised at Farley Hill, by which I infer it is of garden origin, and not *ferae naturae*."

Moore (1966a) reported the following chain of events, beginning at Farley Hill: Sir Briggs gave material of the Farley fern to his friend, T. D. Hill, Esq., of London. Hill presented "the magnificent specimen exhibited at South Ken-

sington" to Col. Miles of Burton Hall, Malmesbury, in the United Kingdom. The gardener there, Mr. J. Green, passed material and information on the fern to Moore. Moore wrote it up as a new species, then apparently learned of the garden origin late in the publication process, added a note saying so, and proceeded with publication of the protologue establishing the species name. The reference to "South Kensington" indicates an exhibition held on July 29, 1865, where the Farley fern won a First Class Certificate (Anonymous, 1865). With these auspicious and well publicized horticultural beginnings, with its novel beauty, and with a tendency toward robust growth, the Farley fern spread through warm-climate and hothouse horticulture to Florida, California, tropical America, and Australia, cropping up in horticultural publications.

An old horticultural question has been whether or not the Farley fern produces fertile spores. Various authors have reported it to be sterile, although Moore described sori and indusia. In 1911, an anonymous author in the Gardener's Chronicle reported "thousands of young plants" raised from the Farley fern with its characteristics nearly carried over to the offspring, except that the fronds were more erect, cold-tolerance was improved, and the characteristic dimorphy between fertile and sterile fronds disappeared, as opposed to having smallish fertile fronds, as Moore had reported. Hoshizaki (1970) observed that, "Early plants were reported sterile, but recent plants are often fertile, and this cultivar comes true from spores." She (pers. comm.) suspects the Farley fern spores to be apogamous. None of the material cultivated at Andromeda Botanic Gardens in Barbados produces spores.

Another horticultural question surrounding 'Farleyense' is its relationship with other *A. tenerum* cultivars. Hoshizaki (1970) listed the following cultivars as descendants of 'Farleyense': 'Fergusonii', 'Gloriosum', and 'Scutum'. Note that the reported approximate 1857 derivation of 'Scutum' is at the very beginning of the likely window of opportunity for the origin of 'Farleyense'. Additional cultivars resemble 'Farleyense', although their precise relationships with it are not known.

Materials and Methods

Herbarium specimens of wild-collected specimens of *A. tenerum* as well as of specimens derived from cultivation at Andromeda Botanic Gardens were prepared and deposited at the Barbados National Herbarium (BAR). Most of the living material at Andromeda Botanic Gardens was studied in situ as well. Some of the herbarium specimens prepared from cultivated specimens were selected deliberately as *A. tenerum* plants having fronds of "normal" morphology mixed with others showing 'Farleyense' morphology. Also studied were herbarium specimens of *A. tenerum*, *A. trapeziforme* L., *A. capillus-veneris* L., and *A. raddianum* C. Presl from the Harvard University Herbaria (A, GH). The Harvard Herbaria were chosen for their well-annotated, rich collections of West Indian ferns. *Adiantum capillus-veneris* and *A. raddianum* were examined because Hoshizaki (1970) noted similarity between these species and *A. tenerum*. In July, 1996, biologist Jeff Chandler and I examined the *A.*

tenerum population growing spontaneously at Farley Hill National Park, but found none suggesting particular similarity to 'Farleyense'. The Sherring specimen of 'Farleyense' from the British Museum was examined from a photocopy of the original.

Morphological Assessment

Moore himself (1866 a, b) was not certain of the direct origin of 'Farleyense' from *Adiantum tenerum*. His words were, "It is probably therefore [referring to the inferred garden origin] a well-marked sport of *A. tenerum,* or it may be a hybrid between *A. tenerum* and *A. trapeziforme,* as it bears a certain degree of resemblance to both." Although it has become commonplace to assign 'Farleyense' to *A. tenerum,* and although *A. tenerum* flourishes at the site of origin, the precise origins of the cultivar remain unknown. Moore's original doubts about its parentage are a bit haunting; cultivated maidenhair ferns in addition to *A. tenerum* could have been at Farley Hill in the 1860s; and (except for Hoshizaki's [1970] generic-level monograph) there has never been a rigorous taxonomic check focused on the relationship between 'Farleyense' and *A. tenerum*—hence the present morphological assessment. This effort was made especially compelling by the presence of hundreds of cultivated and untended specimens of *A. tenerum* at Andromeda Botanic Gardens in Barbados, as well as by frequent observations of wild *A. tenerum* throughout much of the island.

Adiantum trapeziforme (Fig. 4) is indigenous to the West Indies and is popular in horticulture; it could have been present among the Briggs plantings. Other than by having unusually large pinnules, however, there is no resemblance between 'Farleyense' and *A. trapeziforme*. *Adiantum trapeziforme* has pinnules with a heavier texture and a distinctive rectangular shape often ending in an acuminate tip not reflected in 'Farleyense'. The pinnules in *A. trapeziforme* are articulate, unlike those in 'Farleyense'. If hybridization with *A. trapeziforme* played a role in the origin of the Farley fern, it is not apparent morphologically.

Morphological study suggests the Farley fern to be a taxonomically unremarkable garden variant of the highly polymorphic *A. tenerum*. Large, broadly ruffled pinnules make 'Farleyense' eye-catching and easy to identify. However, all of the defining features show up in other combinations in non-'Farleyense' *A. tenerum*. Within *A. tenerum,* different variants featuring different combinations of characters could just as well be propagated and assigned cultivar names if they were of equal garden interest. Moore (1866a) distinguished the Farley fern from other *A. tenerum* as follows, paraphrased. 'Farleyense' tends more toward: Larger pinnules [they tend to be especially broadened and fan-shaped]; sterile pinnules having "dichotomo-laciniate" (vs. less deeply cut) margins; sterile pinnules having entire margins (vs. being denticulate at the vein tips); sterile pinnules strongly differentiated from the fertile pinnules; and young fronds pinkish. Additionally (not from Moore), 'Farleyense' has crisped pinnule margins and particularly droopy fronds.

By far the most conspicuous difference separating 'Farleyense' from most

Herb. Brasil. Regnell. Musei bot. Stockholm.
Plantæ Itineris Regnelliani III. N:o 6752.
Adiantum trapeziforme L.
det. H.H. Maion 1924.
Cuba: prov. Oriente: Sierra de Micara,
prope Mayari arriba, in silvis.
4
3. 3. 1916.
leg. E. L. Ekman.

FIG. 4. *Adiantum trapeziforme* (*Ekman 6752*, GH).

other *A. tenerum* specimens is the large size, fan shape, and deep marginal laciniation of the sterile pinnules. Yet, a systematic check of large numbers of living cultivated plants shows sterile pinnules on *A. tenerum* frequently to approach or equal those of 'Farleyense' in size, degree of marginal dissection, and shape (compare Figs. 5, 6, of 'Farleyense' with Figs. 7, 8, of *A. tenerum*). The large-pinnuled fronds of *A. tenerum* resembling those of 'Farleyense' occur mixed on the same rhizomes with others having smaller pinnules. The

FIGS. 5, 6. Frond details from specimens of 'Farleyense'. 5) Probably derived from close descendant of original plant, or from that plant itself (photocopied image of *Sherring* s.n., BM). 6) From plant cultivated at Andromeda Botanic Gardens *(Rogers 96-100,* BAR).

extreme range of pinnule shape and size in *A. tenerum* warrants emphasis. On different specimens, pinnules may be: longer than broad (e.g., *Wilson 318, Zanoni et al. 21293*), broader than long (e.g., *Maxon 10335*), or extremely small (e.g., *Maxon 10509*). Pink coloration in young fronds turns up as frequently in *A. tenerum* as it does in 'Farleyense' (e.g., *Rogers 96–107, 96–108*).

The distinctive feature of 'Farleyense' that most nearly consistently differentiates it from other *A. tenerum* specimens is the least conspicuous: nearly entire (vs. denticulate) sterile pinnule margins. This feature is truly rare in *A. tenerum,* although it does occur (*Rogers 96-34* on part of a frond, *Maxon & Killip 1670, Swartz 313, Proctor 17296*). The other extreme, with large serrulations, occurs in *L'Herminier* s.n.

Dimorphism between fertile and sterile fronds is as true of *A. tenerum* as of 'Farleyense'. Dimorphism is easier to spot on living material than on herbarium specimens, probably because collectors prefer fertile material. Diminishing further the distinctness of 'Farleyense', Hoshizaki (1970) commented on the "continuously variable" nature of the 'Farleyense' foliar characters and their susceptibility to cultural practices. Within my experience, the specimens of *A.*

FIGS. 7, 8. Frond details from specimens of *Adiantum tenerum* cultivated at Andromeda Botanic Gardens. 7) *Rogers 96-110* (BAR). 8) *Rogers 96-109* (BAR).

tenerum having fronds most similar to 'Farleyense' are cultivated[1]. Undermining the distinctiveness of 'Farleyense' further still is the observation that large-pinnuled variants occur in *Adiantum* species other than *A. tenerum* (Hoshizaki, 1970).

That 'Farleyense' morphology occurs sporadically as odd fronds on otherwise non-'Farleyense' individuals and that similar morphology crops up in other species, hints at the possibility of multiple origins for 'Farleyense'. The anonymous 1911 author plausibly wondered if the fertile Farley fern featured

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[1] Readers may wonder if *A. tenerum* cultivated at Andromeda Botanic Gardens has hybridized with 'Farleyense' to yield the cultivated *A. tenerum* specimens with fronds approaching those of 'Farleyense'. The reasons such hybridization is regarded as unlikely are that: there are hundreds of *A. tenerum* specimens at Andromeda Botanic Gardens scattered over a wide area, and the tendency to form a mix of small-pinnuled and large-pinnuled fronds shows up throughout the grounds. Yet there are only about four 'Farleyense' specimens at the facility; they are non-sporulating and confined to one site. No other *Adiantum* species are present. All of the Andromeda Botanic Gardens specimens that produce fronds resembling 'Farleyense' also produce "normal" *A. tenerum* fronds. That is, individual plants frequently show a mix of the two frond types. Moreover, the tendencies described for Andromeda materials show up in wild *A. tenerum* as well, although not nearly as strongly developed. It is reasonable to suspect that fertilization, manured soil, certain sun or shade conditions, and abundant watering favor development of more or less 'Farleyense'-type fronds.

in that publication could have arisen independently from *A. tenerum*. The 'Farleyense' morphology is speculatively interpretable as the result of a comparatively simple constellation of genetic circumstances captured and disseminated by human-mediated vegetative propagation. It may reflect an adaptive tendency in *A. tenerum* toward broad phenotypic variability. The morphology is characteristic of no wild population, and it is not the outcome of long-term speciation.

In sum, an experienced tropical fern grower has little trouble recognizing 'Farleyense' mainly by its large, fan-shaped, droopy pinnules plus some more subtle characteristics. Yet the foliar features that define the cultivar and other extremes crop up in *A. tenerum*. The most salient character, the large pinnules, occurs mixed with small-pinnuled fronds on many cultivated *A. tenerum* specimens. Foliar variation is extreme in *A. tenerum*. That of 'Farleyense' is attractive and eye-catching, but taxonomically unremarkable, seen in the overall pattern of variation. All evidence points to a human-derived (or human-propagated), horticulturally significant, cultivar whose morphological best fit is with *A. tenerum*. Potential questions for further research on the affinities of 'Farleyense' include the possibilities of polyploidy, multiple origins, and genetic contribution from species other than *A. tenerum*.

TAXONOMY

Moore (1866a) named the Farley fern as a species, which has been followed by some authors (Bailey, 1900; Coulter, 1968). Similarly, other authors or editors (e.g., Synge, 1974; Proctor, 1977; Anonymous, 1996; Leach, 1997) have adopted Andre's combination *Adiantum tenerum* var. *farleyense*. In modern taxonomy, species and varieties are evolutionary products made up of wild populations. Because the Farley fern has no wild populations and is obviously of garden origin (or perhaps a sport removed from the wild into garden propagation), it falls squarely into the category of cultivar, as Hoshizaki (1970) and most others have treated it. That 'Farleyense' fits within the range of variation in *A. tenerum* receives new support from the present work.

NOMENCLATURAL SUMMARY.—The following nomenclatural summary may be of use for future authors having to deal with this group (for a formal description of 'Farleyense', see Hoshizaki [1970].)

Adiantum tenerum Sw., Prodr. 135. 1788 cv.

'FARLEYENSE'.—*Adiantum farleyense* T. Moore, J. Roy. Hort. Soc. 1:82. 1866. *A. tenerum* var. *farleyense* (T. Moore) Andre, Ill. Hort. 19:64. 1872.—NEOTYPE (selected here): illustration of *Adiantum farleyense* in J. Roy. Hort. Soc. [Extracts from the Proceedings of the Royal Horticultural Society] i:xxxv. 1866. [Reproduced as Fig. 9 in the present paper]

TYPIFICATION OF *ADIANTUM FARLEYENSE* T. MOORE.—In the protologue, Moore mentioned basing the name on material obtained from a Mr. Green, as recount-

***Adiantum Farleyense*, Moore.**—Few plants attracted more attention at the late International Exhibition than the truly magnificent specimen of this beautiful fern. The woodcut exhibiting the form of the pinnules was unfortunately not ready when Mr. Moore's paper was published (page 82 of this Journal). The omission is now supplied.

FIG. 9. Neotype of *Adiantum farleyense* and standard for *Adiantum tenerum* cv. 'Farleyense'. Reproduced from the woodcut published later the same year in the same periodical as the protologue (see discussion in text).

ed above. Herbarium specimens from this material are not known, and Moore did not cite a type. Two competing elements are of particular interest as potential types.

Shortly after publishing the protologue (Moore, 1866a), The Journal of the Royal Horticultural Society published four illustrations of 'Farleyense' pinnules, noting. "The woodcut exhibiting the form of the pinnules was unfortunately not ready when Mr. Moore's paper was published (page 82 of this journal). The omission is now supplied." In a subsequent republication of the protologue, slightly emended, Moore (1866b) cited the belatedly published illustrations. They are thus very closely linked to the protologue. The sequence of the three 1866 publications is evident. The protologue was published first. The woodcuts were published explicitly as a late addendum to the protologue shortly on its heels—second in the sequence. The item cited in the present work as Moore (1866b) made reference to the protologue and to the woodcuts, and thus is taken to have come third.

The second potential type is an herbarium sheet at the British Museum labeled "Original Plant, Farley Hill, Barbados 1886, Herbarium of R. V. Sherring" (Fig. 5). This specimen has been anonymously and erroneously annotated (Fig. 10) "isotype specimen of *Adiantum farleyense* Moore in Gard. Chron. 1866: 730. (1866)," (this citation refers to the amended republication of the protologue designated as Moore [1866b] in the present work).

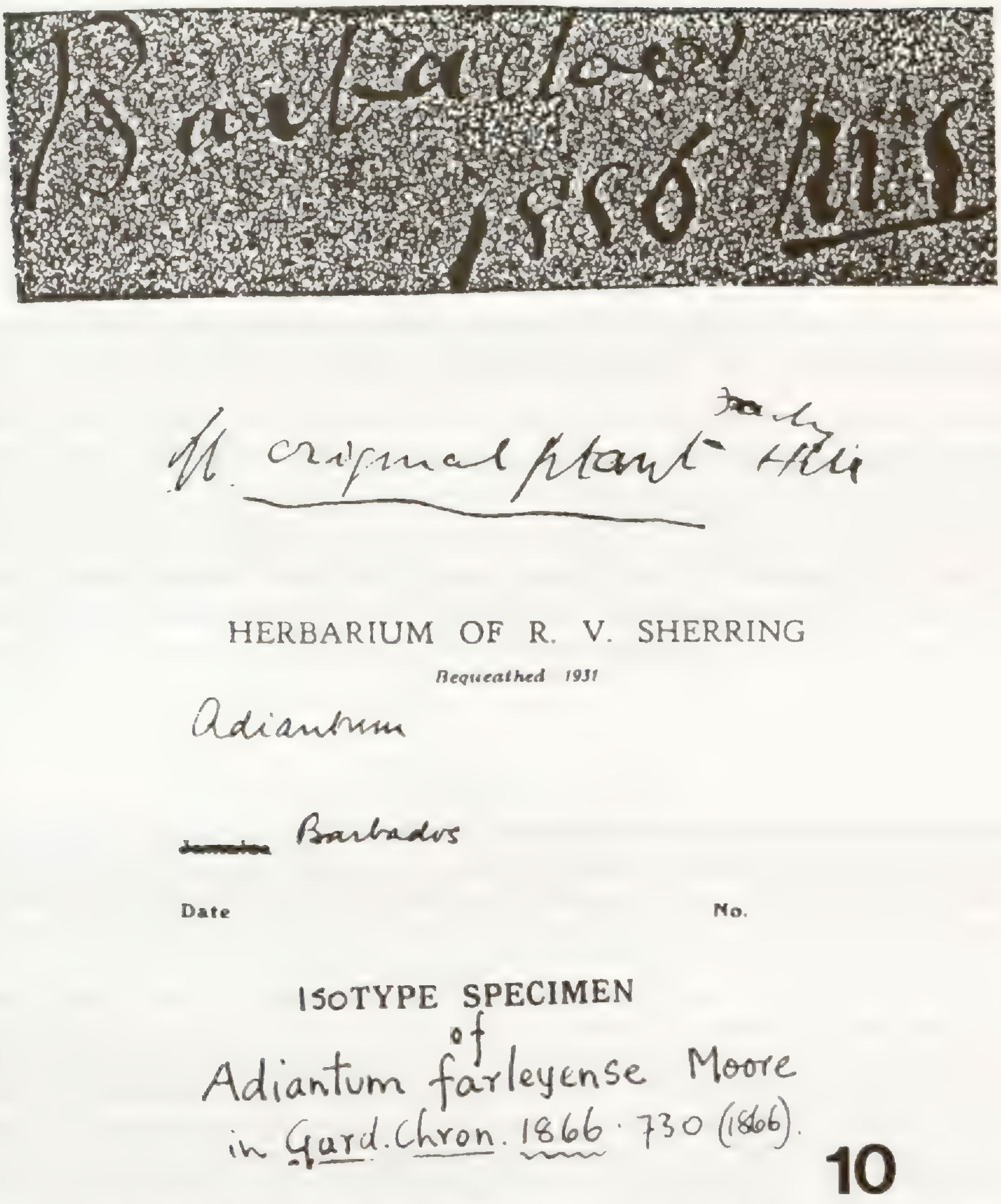

FIG. 10. Labels on *Sherring* s.n. (BM). The designation as "isotype" is erroneous, as is the protologue citation.

The Sherring specimen may come from the same origins as the material behind Moore's protologue, but there is no indication of this in Moore (1866a). If it did come from the original plant, the collection is dated from 20 years after Moore's publication. Not being certain original material, it is not an isotype as defined in Art. 9.3 of the current International Code of Botanical Nomenclature (Greuter et al., 1994).

The element most closely linked with the protologue is the set of belatedly published illustrations, as these had been intended for publication with the protologue (Moore, 1866a), were published only a little later in the same journal, and were cited by Moore (1866b) upon republication of the protologue. Consequently, these figures are selected to neotypify *Adiantum farleyense* in

accordance with ICBN Articles 8.3, 9.6, and 9.9. The figures do not quite qualify as a lectotype, because, according to Art. 9.9 and Footnote 1 (p. 11), a lectotype must come from, ". . . those specimens and illustrations (both unpublished and published either prior to or together with the protologue) upon which it can be shown that the description or diagnosis validating the name was based." The illustrations in question were published (just barely) after the protologue. Nor can it be shown that the protologue was based upon them. Rather, the protologue and figures almost certainly had a common basis in now-missing plant material. The figures are reproduced as Fig. 9 in the present work.

NOMENCLATURAL STANDARD FOR *ADIANTUM TENERUM* CV. 'FARLEYENSE'.—As a cultivar name, 'Farleyense' may appropriately have a standard designated. In accordance with the International Code of Nomenclature for Cultivated Plants, Art. 32.2 (Trehane et al., 1995), the illustration of *Adiantum farleyense* in J. Roy. Hort. Soc. [Excerpts of the Proceedings of the Royal Horticultural Society] i:xxxv, 1866, is here designated as the standard for *Adiantum tenerum* cv. 'Farleyense'. Hence the standard and the neotype are the same set of illustrations (Fig. 9).

ACKNOWLEDGMENTS

Jeff Chandler of the University of the West Indies provided a copy of his conservation assessment of the Farley fern (an unpublished internal document) as well as his substantial files on the fern. He joined me on the field trip to Farley Hill National Park. The Harvard University Herbaria (A, GH) and University of the West Indies Herbarium (BAR) provided herbarium specimens. The curators at Harvard, especially Emily Wood, are thanked for helping with the loan. Also appreciated was permission from the British Museum (to J. Chandler during a visit) to photocopy the Sherring specimen of 'Farleyense'. Access to the Barbados National Trust's Andromeda Botanic Gardens has been critical to the study, and the Trust is acknowledged for encouraging this and other research. Barbara Joe Hoshizaki, Alan Lievens, John Mickel, and Robbin Moran generously provided thoughts and advice through correspondence. The Los Angeles International Fern Society attempted to find materials of 'Farleyense' by placing a plea in their journal. Thanks are due Audrey Thomas of "Growing Things" for donating living plants of the Farley fern to Andromeda Botanic Gardens and for allowing me to examine a magnificent specimen of 'Farleyense' at her home. The Barbados National Conservation Commission granted permission to search Farley Hill National Park for adiantums of interest.

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APPENDIX: SPECIMENS EXAMINED

Adiantum capillus-veneris: **Bermuda.** *Baker 656* (A-cultivated). **Cuba.** *Jack 7964* (GH); *Graves 1432* (GH); *Tryon & Tryon 5584* (GH). **Haiti.** *Ekman 7753* (GH); *Leonard 3780* (GH). **Jamaica.** *Moore* s.n. (GH). **Puerto Rico.** *Scamman 6555* (GH); *Wayne 1103* (A-cultivated).

Adiantum raddianum: **Bermuda.** *Baker 619* (A-cultivated). **Dominica.** *Hodge 69* (GH). **Haiti.** *Judd 3803* (GH). **Jamaica.** *Maxon 10274* (GH); *Proctor 22949* (GH).

Adiantum tenerum (of cultivated origin): **Barbados.** Andromeda Botanic Gardens, *Rogers 96-34* (BAR), *Rogers 96-35* (BAR), *Rogers 96-36* (BAR), *Rogers 96-37* (BAR), *Rogers 96-102* (BAR), *Rogers 96-103* (BAR), *Rogers 96-104* (BAR), *Rogers 96-105* (BAR), *Rogers 96-107* (BAR), *Rogers 96-108* (BAR), *Rogers 96-109* (BAR), *Rogers 96-110* (BAR).

Adiantum tenerum (of wild origin): **Bahamas.** *Bryant* s.n. (GH); *Gillis 7392* (A); *Sauleda & Correll 2210* (GH); *Wight 185* (GH); *Wunderlin et al. 8310* (GH). **Barbados.** *Proctor 25841* (A); *Rogers 96-1* (BAR), *Rogers 96-2* (BAR), *Rogers 96-111* (BAR). **Cuba.** *Abarca 4502* (GH); *Curtiss 590* (GH); *Shafer 1259* (GH), *Shafer 3542* (GH); *Wilson 318* (GH). **Dominica.** *Hill 21336* (GH); *Hodge & Hodge 1645* (GH), *Hodge & Hodge 1827* (GH). **Dominican Republic.** *Abbott 1142* (GH), *Abbott 1644* (GH); *Jones & Norris 1134* (GH), *Jones & Norris 1146* (GH); *Zanoni et al. 19745* (GH), *Zanoni et al. 21293* (GH), *Zanoni et al. 21502* (GH). **Grenada.** *Proctor 17296* (A). **Guadeloupe.** *L'Herminier* s.n. (GH); *Le Gallo 575* (A); *Proctor 19965* (A); *Scamman 8161* (GH). **Jamaica.** *Gilbert 162* (GH), *Gilbert 162bis* (GH); *Howard & Proctor 14035* (A); *Maxon 10335* (GH), *Maxon 10509* (GH); *Maxon & Killip 1670*

(GH); *Millspaugh 905* (GH), *Millspaugh 1900* (GH); *Swartz 313* (GH); *Wilson 521* (A). **Montserrat.** *Proctor 1959* (A). **Nevis.** *Proctor 19453* (A). **Puerto Rico.** *Britton & Cowell 2010* (GH); *Garber 140* (GH); *Kuhn 46* (GH); *Stimson 3553* (GH). **St. Croix.** *Proctor 20458* (A); *Ricksecken 472* (GH). **St. Martin.** *Proctor 18722* (A).

***Adiantum tenerum* cv. 'Farleyense'** (of cultivated origin): **Barbados.** "Original plant", Farley Hill, *Sherring* s.n. (BM, photocopy examined); Andromeda Botanic Gardens, Rogers 96-100 (BAR), *Rogers 96-101* (BAR).

Adiantum trapeziforme: **Cuba.** *Brues* s.n. (GH); *Ekman 6752* (GH); *Morton 4415* (GH); *Shafer 3974* (GH). **Jamaica.** *Crosby et al. 198* (GH).

American Fern Journal 88(1):47–48 (1998)

Shorter Note

Japanese Beetle Attacks Bracken in Vermont.—Bracken, *Pteridium aquilinum* (L.) Kuhn, supports a relatively rich insect fauna, with more than 100 species reported to feed on it (Balick et al., Oecologia 35:55–89, 1978). Many or most are host-specific specialists, ranging from moths (Lepidoptera: Noctuidae) to sawflies (Hymenoptera: Tenthrendinidae) and aphids (Homoptera: Aphididae), which bore into the rhizomes, mine the leaves, or feed on soft parts. Relatively few are generalist feeders on the laminar surfaces, perhaps in part because ants defend nectaries at the junctures of the rachis and costae on young fronds, killing or driving off potential competitors for nectar. Cooper-Driver (Ann. Missouri Bot. Gard. 77:281–286, 1990) hypothesized that ants are a significant natural force selecting against generalists.

Later in the season, when ants are absent, bracken may be more susceptible to herbivory by generalists. However, chemical defense in the form of condensed tannins, which render the plants unpalatable and bind proteins when ingested, is high.

Very few beetles (Coleoptera) are known to feed on bracken. In a summary of taxonomic status and host specialization of 27 insects that regularly feed on bracken in Britain, none were found to be beetles. In Papua New Guinea, 16 species were noted (Lawton and MacGavin, Proc. Roy. Soc. Edinburgh 86B: 125–131, 1985). Lawton (Bot. J. Linn. Soc. 73:187–261, 1976) also noted a scarab beetle, the garden chafer, *Phyllopertha horticola* L., on bracken. In two years of observations in Natick, Massachusetts, Cooper-Driver collected a number of adult beetles from bracken, but these were scavengers rather than herbivores. They included click beetles (Elateridae), checkered beetles (Cleridae), false click beetles (Eucnemidae), and scarab beetles (Scarabaeidae). Japanese beetle, *Popillia japonica* Newm., a scarab beetle and a generalist feeder, was observed once (21 July 1980), but no damage to the plant was observed. Except for an old "list of plants often attacked" reported in the agricultural literature (Bailey, *The Japanese beetle in Vermont,* Vermont. Agric. Dept. Bull. 5, 1940), we have found no previous report of Japanese beetle feeding on bracken.

On 14 August 1996, Gilman observed a heavy infestation of adult Japanese beetles on northern bracken, *P. aquilinum* var. *latiusculum* (Desv.) Underw., near Twinfield High School, Marshfield, Washington County, Vermont. Japanese beetles were first introduced into the United States in ca. 1916 (C.S. Elton, *The ecology of invasions by animals and plants,* Chapman and Hall, 1958) and are now a serious pest of agricultural and ornamental plants. Adults feed on foliage and flowers of a variety of angiosperm families, perhaps most conspicuously on Rosaceae. According to Bailey (*The Japanese beetle in Vermont,* Vermont. Agric. Dept. Bull. 5, 1940) Japanese beetles reached Vermont in 1932, but they have invaded this northern section of the state only within the past decade (Gilman, pers. obs.). They are now conspicuous in mid-to late summer

FIG. 1. Partial skeletonization of northern bracken (*Pteridium aquilinum* var. *latiusculum*) by Japanese beetle (*Popillia japonica*). Veins, costules, and segment margins remain intact; damage is confined to central portions of the frond.

in gardens and fields, but are confined largely to settled areas and river valleys, where there are suitable areas of grass turf (e.g., lawns and pastures) to support the larvae, which feed primarily on grass roots. The Marshfield site is on a sandy terrace between the school soccer field and the Winooski River at an elevation of ca. 235 m. Associated plants are, among others, Hungarian brome, *Bromus inermis* Leyss., and scouring-rush, *Equisetum hyemale* L. Similar infestations were found subsequently in several other local populations.

The damage caused by Japanese beetles to bracken is distinctive (Fig. 1). It is confined primarily to the central portions of mature fronds. Tissue is selectively eaten between the veins, partially skeletonizing the lamina. The veins, costule, and segment margin remain largely intact. It is possible that the segment margin, because it is revolute and thickened with a false indusium, is too thick or tough to be eaten. However, younger tissues toward the tips of the pinnae, presumably thinner and less tough, also are not eaten. Subsequent to the removal of some of the laminar tissue, much of the remaining tissue becomes necrotic and brown, giving the damaged frond a characteristic aspect. Specimens of representative damaged fronds (*Gilman 96232*) have been deposited at BU, VT and NEBC.—ARTHUR V. GILMAN, P.O. Box 82, Marshfield, VT 05658, and GILLIAN COOPER-DRIVER, Department of Biology, Boston University, 5 Cunningham Street, Boston, MA 02215.

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American Fern Journal 88(2):49–59 (1998)

Cyathea planadae, a Remarkable New Creeping Tree Fern from Colombia, South America

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ABSTRACT.—A new species within the Cyathaceae is described from the mid-elevation cloud forests of the Andes in southwestern Colombia. *Cyathea planadae* N.C. Arens & A.R. Smith has fragile, ephemeral, sphaeropteroid indusia surrounding inframedial to medial sori, which are typical of many *Cyathea*; however it is unusual in possessing a creeping stem. Decumbent stems (above-ground rhizomes) bearing adventitious roots and buds give rise to narrow, glabrous upright shoots upon which three or four twice-pinnate-pinnatified fronds are typically displayed. *Cyathea planadae* is very common and patchily distributed in the understory of primary forest habitats in montane forests. The species is also noteworthy for an apparent association with an ant species, which appears to feed from pads of tissue at the base of the pinnae.

Tree ferns of the family Cyatheaceae are a common, abundant, and ecologically important part of the flora of montane and premontane wet forests in the Andes of Colombia. Taxonomic note of this group was first made in the mid eighteenth century with initial floristic surveys of the Andes by Spanish botanists such as José Celestino Mutis (Díaz-Piedrahita, 1991). A taxonomic revision of *Cyathea* and related genera in the Andes was undertaken in the 1960s and 1970s by Rolla Tryon and his students. Recent and ongoing studies by Robbin Moran in Ecuador and Central America (Moran, 1991, 1995) have revealed additional tree fern species richness in montane habitats, including many taxa that are ecologically restricted to the understory of primary forests. Ecological studies of the distribution of tree ferns across the successional mosaic at La Reserva Natural La Planada in southwestern Colombia led Arens to investigate members of the Cyatheaceae found in an Andean forest, relatively undisturbed by human activity. This, in turn, led to the recognition of this unusual new species.

La Reserva Natural La Planada, 1°09′37″N, 77°59′13″W, 1,850–2,300 m elevation, is located in the Colombian department of Nariño near the town of Ricaurte (Fig. 1). La Planada consists of 3,200 hectares of privately owned reserve on the Pacific slope of the Andes and is part of the Chocó biogeographic province. Mean annual rainfall averages 4,500 mm, with a drier period in July and August; average daily temperature is 19°C. Natural vegetation within the reserve is typical of Andean cloud forest, with a canopy of approximately 25 m in height composed of relatively few woody angiosperm species. The most common canopy trees include *Alchornea* (Euphorbiaceae), *Clusia* (Guttiferae), *Inga* (Fabaceae), *Miconia* (Melastomataceae), *Myrica* (Myricaceae), and *Psidium* (Myrtaceae) and *Otoba* (Myristicaceae). The reserve includes primary

forest, secondary forest, and recently abandoned pasture on both flat and steep-slope topography.

The Andean region, which includes montane areas of Colombia, Ecuador, Venezuela, Peru, Bolivia, and contiguous lowlands of the Chocó biogeographic province, is an important center for species diversity and endemism in ferns (Tryon, 1972; Lellinger, 1975; Tryon and Gastony, 1975). This pattern is particularly pronounced in *Cyathea* (sensu Lellinger, 1987) and *Cnemidaria* (Stolze, 1974; Tryon, 1976). The Andean regional center (Tryon, 1972) harbors a significant number of narrowly endemic, ecologically restricted, and commonly poorly collected tree fern species including *Cyathea decorata* (Maxon) R.M. Tryon (Colombia, Valle del Cauca); *Cyathea phalaenolepis* (C. Chr.) Domin (Colombia, Valle del Cauca; Ecuador, Esmeraldas); *Cyathea latevagans* (Baker) R.M. Tryon (Colombia, Antioquia, Chocó; Ecuador, Carchi); *Cyathea hemiepiphytica* R.C. Moran (Ecuador, Esmeraldas, Carchi; Colombia, Chocó); *Cyathea parva* (Maxon) R.M. Tryon (Colombia, Valle del Cauca); *Cyathea haughtii* (Maxon) R.M. Tryon (Colombia, Santander; Ecuador, Zamora-Chinchipe); *Cyathea alstonii* R.M. Tryon (Colombia, Meta); *Cyathea peladensis* (Hieron.) Domin (Colombia, Huila, Cauca, Putumayo); *Cnemidaria tryoniana* Stolze (Colombia, Antioquia); *Cnemidaria singularis* Stolze (Colombia, Putumayo); *Cnemidaria chocoensis* Stolze (Colombia, Chocó); *Cyathea impar* R.M. Tryon (Panama, C. Jefe); and *Cyathea falcata* (Kuhn) Domin (Panama; Colombia, Chocó).

Cyathea planadae N.C. Arens & A.R. Smith, sp. nov.—TYPE: Colombia, Nariño, Reserva Natural La Planada, ca. 1°09′37″N, 77°59′13″W, large plants in forest undisturbed by human activity, 1,850 to 2,300 m, *N. C. Arens* s.n. on 16 July 1995 (UC; isotypes COL, NY).

Differt ab aliis speciebus *Cyatheae* rhizomatibus decumbentibus, rhizomatibus ad extremum ascendentibus et truncos erectos parientibus, truncis 0.2–5 m longis, 2.5–5 cm diam., viridulis, praeter apices glabris et plus minusve nitidis, maximam partem radicibus adventitiis carentibus autem adventitiis gemmis vel caulibus brevibus praeditis, plantis colonialibus, paleis paucis adpressis limitatis ad apicem caulium et basi stipitum, bicoloribus, non conformibus, ca. 4–6 × 1.5–4 mm, atrobrunneis centraliter, ad marginem fulvis vel stramineis fimbriatis vel ciliatis, frondibus 3–8 cm distantibus, pinnis pinnulisque ad basim aerophoris nigrescentibus prominentibus, elevatis, glabris, ut videtur formicis consociatis. Laminae bipinnatae-pinnatifidae; venae furcatae; sori inframediani vel mediani; indusia membranacea, sphaerica, fragilia, evanescentia, in cupulas vel fragmenta cupularum fatiscentia.

Decumbent and upright stems morphologically and anatomically indistinguishable except for the greater abundance of adventitious roots on the prostrate portion (Fig. 2). Adventitious buds present and sometimes developing on both upright and horizontal portions of the stem. Decumbent stems long-creeping, producing adventitious roots; decumbent stem apices ultimately ascending and producing trunks 0.2–5 m tall, 2.5–5 cm diameter; decumbent and

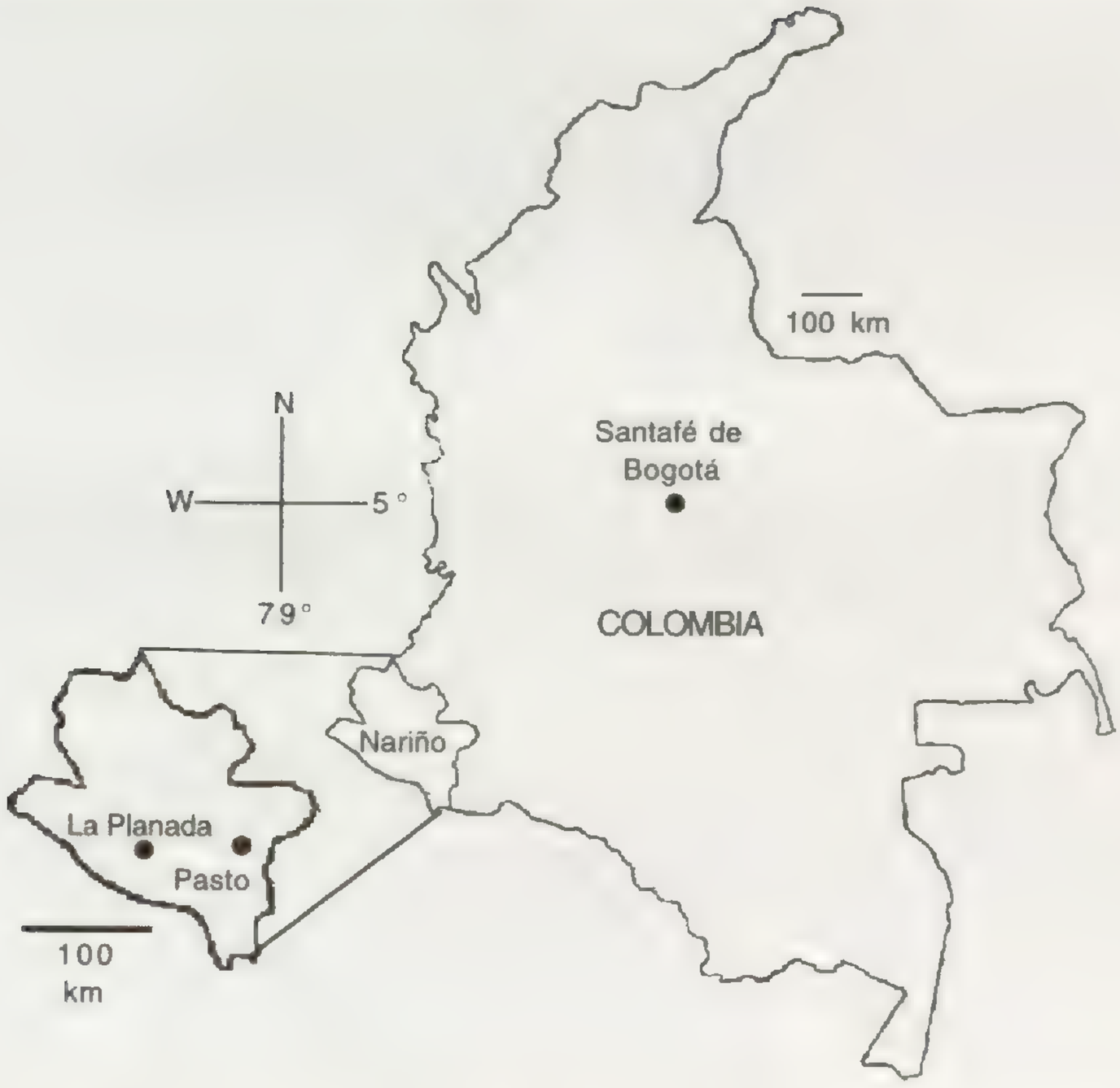

FIG. 1. Location of La Reserva Natural La Planada in Colombia.

upright stems greenish, more or less glabrous, the adventitious buds developing into leaf-producing meristems if the growing apices are damaged, the plants forming colonies. Fronds 50–150 cm long, arising at intervals of 4–9 cm on erect stems, abscising cleanly and leaving leaf scars, which are also visible on decumbent stems, at intervals of 3–8 cm. Petioles stramineous to tan, 25–55 cm long, 1–2 cm diameter at the base, spiny, the spines 2–3 mm long × 1.0–1.5 mm thick at their bases; scurf (squamellae) present at base, stramineous to tan, ca. 1–1.5 mm long, with marginal and, especially, basal projections, a few of the squamellae brown-streaked; petiole scales very dark brown centrally, sometimes irregularly black-streaked toward tip, with tan to stramineous fimbriate or ciliate margins (scales marginate and non-conform in the terminology of Tryon, 1976), indurate, mostly 4–6 × 1.5–4 mm, tightly appressed on croziers. Blades 2-pinnate-pinnatifid, 50–150 × 40–120 cm, truncate at base, gradually narrowed distally to the apex; pinnae ca. 12 pairs, alternate, the largest ca. 60 × 13 cm, at their base with prominent darkened, raised, glabrous pads (aerophores), pads visited and apparently chewed upon by ants; pinnules up to ca. 8 × 17 cm, sessile or the largest stalked to 1 mm, the sides nearly parallel for ¾ of their length, then tapering to the acuminate tip, incised to ca. 0.5–1 mm from costule, at their base with darkened pads similar to those at the base of the pinnae, but smaller, ca. 0.5–0.6 mm long; ultimate segments 3–4 mm wide, 12–15 pairs per pinnule, entire; rachises, costae, and costules stramineous, with dark brown, mostly appressed, ovate to lanceolate, flattish scales mostly 1–2 mm long, lacking hairs; rachises and costae also with persistent trichomidia (highly dissected, whitish or hyaline scales appearing al-

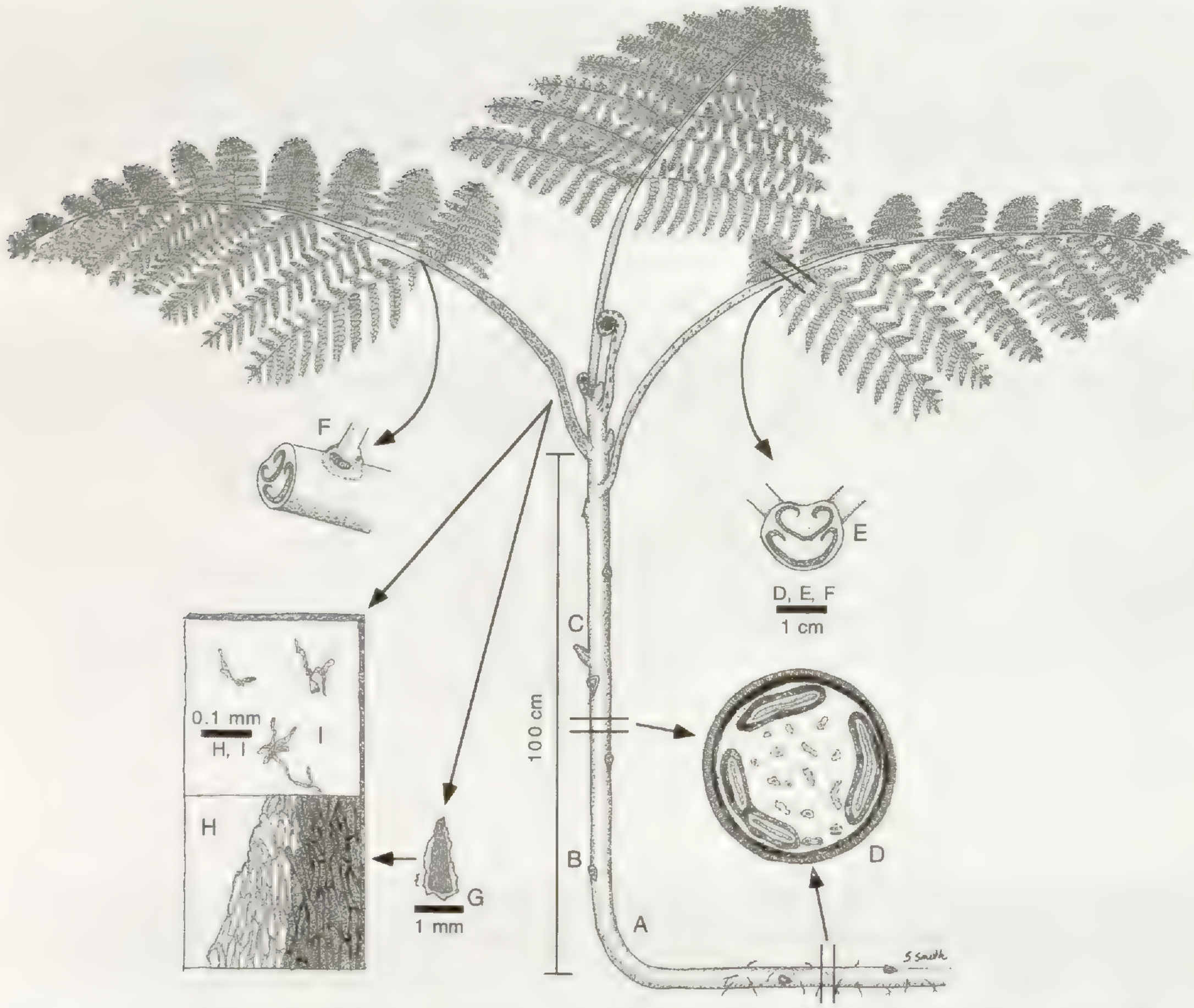

FIG. 2. Growth habit and some anatomical and morphological features of *Cyathea planadae*. A) growth habit of the sporophyte showing decumbent and erect stem orientation; B) raised leaf scars; C) adventitious buds; D) cross section of stem showing vascular bundles close to the axis margin; E) cross section of petiole taken at the first primary pinnae showing two c-shaped vascular traces and the orientation of primary pinnae; F) point of attachment of pinna to the rachis showing pads on which ants were observed; G) petiole scale; H) petiole scale margin; I) petiole scurf.

most hairlike; veins 1-forked to simple near segment tips, the forks inframedial to medial, 7–9 pairs (below fork) per segment; laminar tissue between veins glabrous. Sori inframedial to medial, borne at the vein fork, indusiate, the indusia sphaeropteroid or nearly sphaeropteroid, fragile, evanescent and fragmenting early, visible only as a basal cup or cup fragments in mature sori; sporangia arising from a prominently raised, conical receptacle; receptacle bearing hyaline paraphyses ca. 0.1 mm long. Spores (Fig. 3) trilete, 25–30 μm in equatorial diameter, tetrahedral in shape with the interradial region slightly concave, psilate with a coarse and irregular verrucate sculpture in the equatorial zone, laesural arms extending two-thirds of the way to the equator of the spores, labra slightly raised and extending approximately 1 μm from the laesura and bordered by a row of pits, rodlets (characteristic of the perine of many *Cyathea* species, Tryon and Lugardon [1991]) absent.

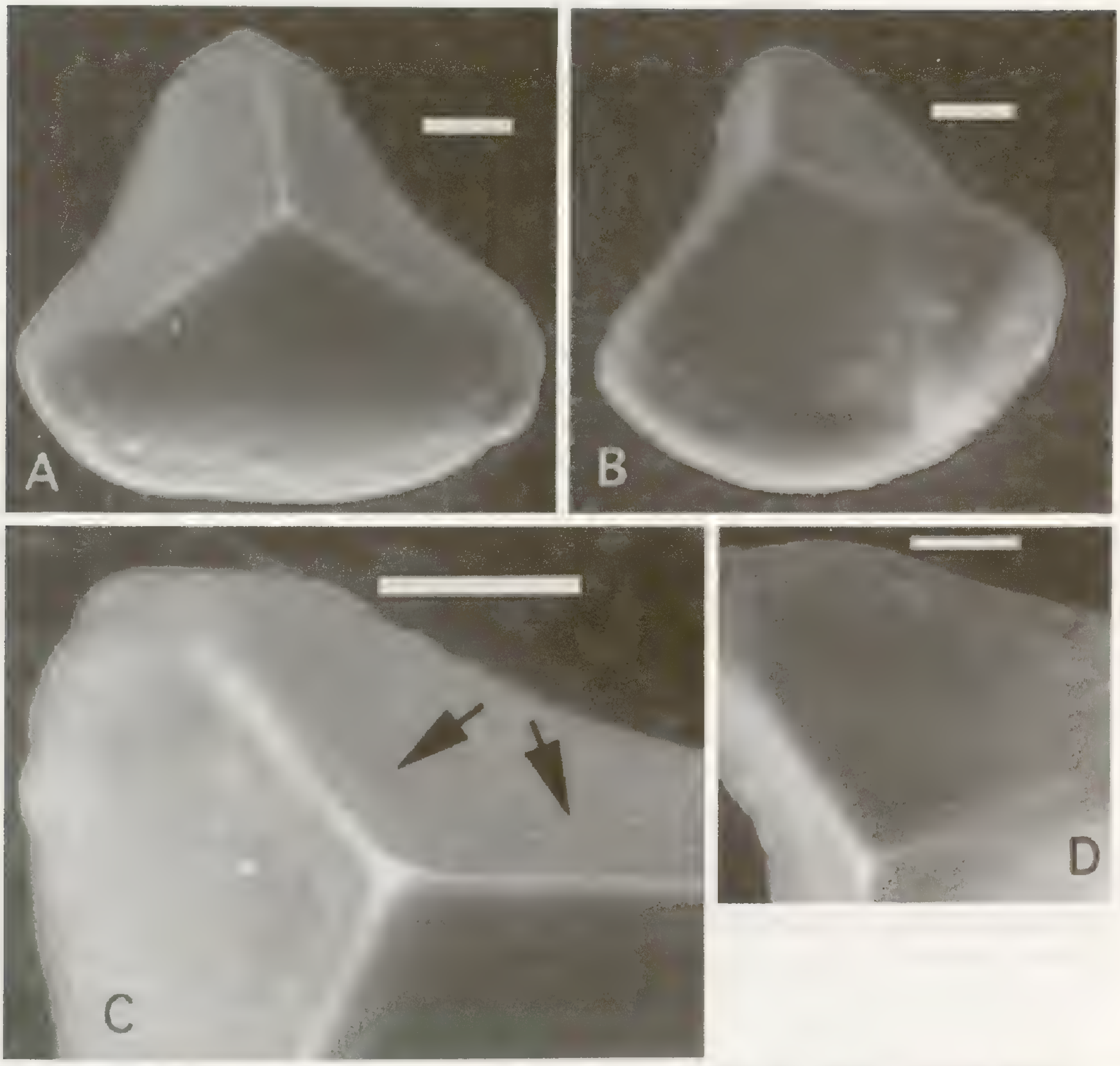

FIG. 3. Electron micrographs of *Cyathea planadae* mature spores. Micrographs were taken of air-dried, untreated spores collected in the field. Images were made using an Environmental SEM in the UC Museum of Paleontology. Untreated spores were mounted on adhesive stubs and imaged at 10 kV under 4.1 torr wet atmosphere. A) Spore of *C. planadae* showing raised laesura; B) spore oriented to highlight equatorial verrucae; C) fine pitting along laesural arms indicated by arrows; D) spore oriented to illustrate verrucate sculpture in equatorial region. Scale bars = 5 μm.

PARATYPES.—COLOMBIA. **Nariño**. La Planada, Salizar Finca, 7 km above Ricaurte, 1°08′N 77°58′W, Transect 3, 1,750 m, *Gentry et al. 35163* (MO); same locality, Transect 2, 1,750 m, *Gentry et al. 35121* (MO); Reserva Natural La Planada, in front of "La Posada", 1°09′37″N, 77°59′13″W, *Bittner 2656* (MO, PSO, UC); Mpio. de Ricaurte, Reserva Natural La Planada, camino al Hondón, 1°09′37″N, 77°59′13″W, 1,850 m, *Guzmán 07* (UC). **Valle de Cauca**. Mpio. Queremal, Campamento Tokio (telecom towers), 9 km west of Queremal; ca. 3°30′N, 76°42′W, 2,000–2,100 m, *Luteyn et al. 12575* (UC). ECUADOR. **Napo**. Cantón, Quijos, Baeza, 00°28′S 77°54′W, 2,200–2,300 m, *Palacios & Freire 4988* (UC).

Cyathea planadae is remarkable for two features: the long, creeping, above-ground, decumbent stems that send up, at intervals, slender trunks; and an apparent association with ants. We refer to the prostrate or creeping portion of the stem as a "decumbent stem" rather than as a "rhizome" because in *C. planadae* this structure is not dorsiventrally flattened as is common in fern

rhizomes and the structure is always above ground. Decumbent stems are anatomically and morphologically indistinguishable from erect stems, and have visible leaf scars, which suggests that these were once upright stems; thus, no morphological distinction between upright and prostrate portions of the stem seems appropriate. Decumbent stems have abundant slender, black adventitious roots that arise from below leaf scars and below adventitious buds; adventitious roots are also common on larger-diameter upright stems. Adventitious buds are visible at intervals below the leaf-producing apical meristem of erect axes; adventitious buds form at random intervals along the stem and are not associated with leaves or leaf scars. Neither erect nor decumbent stems branch at the apex; adventitious buds remain inactive while the leaf-producing apical meristems grow and produce leaves. However, when the leaf-producing apical meristem of a stem is damaged and no longer produces leaves, adventitious buds become active and send up new vertical trunks. Adventitious buds also are important in the creeping habit of the species; when an erect axis falls and becomes decumbent, the apical meristem ceases to produce leaves and adventitious buds activate to initiate new upright stems. Prostrate stems could be followed for 2–7 meters through the leaf litter and soil O-horizon, and commonly terminate in a broken or rotted end distal to the active portion of the clone. This observation suggests that the decumbent stems may have a clonal history beyond that evidenced by the stems observed. Young sporophytes appear to creep for up to 30 cm before giving rise to upright, frond-bearing stems. In very small (young) plants (upright height less than 30 cm), decumbent stems may support up to three or four upright stems, each arising from a separate adventitious bud; small, upright stems may be as little as 10 cm apart on decumbent stems. Later, multiple upright stems apparently self-thin to a single, active, apical meristem.

Clonal growth was reported in the Brazilian species *C. sampaioana* [= *Alsophila sternbergii* (Sternb.) Conant] (Brade, 1971). In this species, adventitious buds were found at the base of the erect trunk of one individual. *Alsophila sternbergii* is not closely related to *C. planadae*. Gastony (1973) noted that this form of vegetative reproduction may be similar to that observed in the African species, *C. manniana* Hook. [= *Alsophila manniana* (Hook.) R.M. Tryon], by Halle (1966). One of us (NCA) also observed sprouting of new trunks from the base of an individual of cf. *Alsophila* at La Planada. However, clonal habit is not known to be a regular growth form of any of these species as it is in *C. planadae*.

Although most tree ferns have a stout vertical trunk and lack long-creeping stems, there are a few species that differ in this character, e.g., *C. assurgens* R.M. Tryon, from Colombia and Ecuador, which has very slender, almost hemiepiphytic stems supported by other vegetation and widely spaced leaves. It differs from *C. planadae* by its pinnate-pinnatifid blades and golden rhizome scales, and is not closely related. Erect stems of *C. planadae* are free-standing, not hemiepiphytic or with a tendency to climb, although some larger erect stems may lean against and be partially supported by surrounding vegetation. Over time, decumbent stems of *C. planadae* may be buried by forest litter to

give the structure an underground appearance that conceals the interconnectedness of erect trunks. This feature may explain why the clonal nature of *C. planadae* has not been previously noted. However, it seems clear that horizontal stem growth begins on the surface or within litter as stems become decumbent in very small plants.

Within undisturbed forest, *C. planadae* is the most common cyatheaceous species at La Planada. Its distribution tends to be patchy; each patch may represent the vegetative spread of a single sexually produced sporophyte. This species is absent from open habitat within the reserve and appears as sporelings less than 20 cm high in closed-canopy secondary forest representing approximately 15 years of regeneration (Arens and Sánchez, 1994, 1998). Other tree fern species known from La Planada are *Alsophila engelii* R.M. Tryon, *A. erinacea* (H. Karst.) D.S. Conant, *Cnemidaria quitensis* (Domin) R.M. Tryon, *Cyathea caracasana* (Klotzsch) Domin, and another undescribed species of *Cyathea* (R. C. Moran, pers. comm., 1997)

A second remarkable feature of *C. planadae* is an apparent association with the ant *Gnamptogenys nigrivitrea* Lattke. We observed ants, generally only one or two individuals at a time, on the surface of fronds. Ants on fronds are commonly inactive or observed with their mandibles piercing glabrous pads at the base of primary pinnae (Fig. 2). Holttum and Edwards (1983) mentioned a similar "swollen dark glossy aerophore" at the base of the pinnae in *C. akawaiorum* Edwards, from Guayana, but this species is not closely related to *C. planadae,* and it is not clear whether the structures described for *C. akawaiorum* are homologous with those of *C. planadae*. However, we have seen darkened pads at the base of the pinnae in herbarium specimens of many tree ferns. The function of aerophores in other groups of ferns is thought to be improved gas exchange within the frond (Smith, 1980). Inactive ants on fronds could not be coaxed to activity by physical disturbance of the leaf. Because observations were made only during the day, we suspect that this species may be nocturnal. We did not observe ant domatia (sensu Hölldobler and Wilson, 1990) produced by the plant or ants creating living space within the petioles, fronds, or stems. However, some dried specimens of *C. planadae* in our collection have many individuals of *Gnamptogenys nigrivitrea* preserved on the fern rachis. Although they are relatively large, these ants were not observed on the frond at the time of collection, leading us to suspect that they were housed inside the rachis and emerged in response to heat-drying after collection.

Aerophores in tree ferns are generally not as prominent as those of *C. planadae*. In *C. planadae* these structures are clearly raised, and the activity of ants is evidenced by bite marks on the pads. *Gnamptogenys nigrivitrea* is reportedly carnivorous (J. E. Lattke, pers. comm., 1996); however, many carnivorous ants are known to supplement their diet with plant liquids (Hölldobler and Wilson, 1990). *Gnamptogenys* is noted to inhabit the internode spaces of *Cecropia* saplings in Costa Rica, Peru, and Brazil, but is never found in mature trees (Longino, 1991).

Pad-like aerophores are also visible at the base of the petioles in three her-

barium specimens (*Bittner 2656, Gentry et al. 35121, Palacios & Freire 4988*), and are visible in field photos of the species. In these specimens, the aerophores are arranged in a semicircular arc where the petiole abuts the erect stem. In the arc, there are 7 aerophores, each ca. 6 × 2 mm. There is also an even larger aerophore ca. 10 × 2 mm just below the attachment of the petiole on the glabrous stem (*Bittner 2656*). However, aerophores at the base of petioles do not show clear evidence of ant activity.

In most documented ant-plant symbioses, the plant partner supplies ants with food, shelter, or both, in exchange for some benefit that may range from protection from herbivores to enhanced nutrient or carbon acquisition to spore dispersal (Tryon, 1985; Hölldobler and Wilson, 1990; Treseder et al., 1995). A variety of ant associations has been observed in ferns. Some fern taxa have morphological features that facilitate ant habitation. The neotropical *Solanopteris brunei* (Wercklé) W.H. Wagner produces hollow rhizomes that house *Azteca* ants (Wagner, 1972; Gómez P., 1974). Several species of the paleotropical genus *Lecanopteris* also produce hollow rhizomes that are commonly colonized by ants (Hennipman and Verduyn, 1987). *Lecanopteris spinosa* Jermy & T.G. Walker is characterized by a highly-developed rhizome with elaborate hollow chambers that are inhabited by ants (Jermy and Walker, 1975). *Lecanopteris mirabilis* (C. Chr.) Copel. produces a series of broad rhizome segments that attach to a substrate, usually a tree trunk, to form external ant domatia (Walker, 1986). *Lecanopteris sinuosa* (Wall. ex Hook.) Copel. produces similar hollow rhizomes and extra-rhizome domatia (Janzen, 1974). In *L. sinuosa,* plants are believed to receive nitrogen and phosphorus from decaying organic matter placed within the rhizome or domatia by ants. Hand-sectioning in the field did not reveal either hollow rhizomes or petioles in *C. planadae,* however further investigation is necessary.

Other fern taxa provide nectaries that supplement the diet of associated ants. Many drynarioid ferns (e.g., species of *Drynaria,* and *Aglaomorpha*) produce nectaries on adult leaves that encourage ant activity. These nectaries appear as patches of cells, lacking chlorophyll, on the frond laminae (Zamora and Vargas, 1974). The position of nectaries relative to costules and costae provides useful taxonomic information (Zamora and Vargas, 1974). Nectaries, which exude sucrose and glucose, were first reported in common bracken, *Pteridium aquilinum* (L.) Kuhn (Bonnier, 1879). Among the Cyatheaceae, nectary-like structures have been reported in *Cyathea arborea* (L.) Sm. and *Hemitelia horrida* (L.) R. Br. [= *Cnemidaria horrida* (L.) C. Presl] (Bonnier, 1879). Sugars and other nutritive substances have been reported in the exudate of fern nectaries by a variety of workers and in a variety of groups: glucose from *Platycerium* (Dümmer, 1911); sucrose, fructose and glucose from *Photinopteris speciosa* (Blume) C. Presl and *Platycerium* spp. (Lüttge, 1961); sucrose, fructose, glucose, and a variety of amino acids from *Polypodium myriolepis* H. Christ, *Polypodium pyrrholepis* (Fée) Maxon, *Polypodium rosei* Maxon, *Polypodium sanctae-rosae* (Maxon) C. Chr., *Polypodium thyssanolepis* A. Br. ex Klotzsch, *Drynaria quercifolia* (L.) J. Sm., *Drynaria rigidula* (Sw.) Bedd., and *Polybotrya osmundacea* Humb. & Bonpl. ex Willd. (Koptur et al., 1982). Koptur and col-

leagues concluded that the presence of sucrose-poor nectar that also contains amino acids in these taxa is unlikely to exist solely as a mechanism to remove waste metabolite from the plant but may have the added function of encouraging the presence of ants, some of which forage for sugar and/or amino acid-rich liquids. In *C. planadae,* we did not observe any extruded droplets of nectar under field conditions, consequently no assay of substances produced by the glabrous pad could be performed. Because ants are always present in the field to consume produced nectar, it may not have the opportunity to accumulate in droplets.

The closest affinities of *C. planadae* are with *Cyathea* sensu Tryon (1976) and with the *Cyathea* clade, sensu Conant et al. (1995). However, the unusual morphology of *C. planadae* suggests that relationship to any known species is remote, and we are unable to ascertain with certainty to which of Tryon's groups *C. planadae* might belong. The sphaeropteroid or nearly sphaeropteroid indusia that completely surround the sori suggest that species 1–19 in Tryon (1976) are not closely related to *C. planadae* because they all have hemitelioid or (spp. 17–19) cyatheoid indusia; several hemitelioid species also are less dissected, having pinnate or bipinnate blades and, in some, a conform apical pinna (the *C. petiolata* (Hook.) R.M. Tryon and *C. speciosa* Willd. groups, spp. 9–16). Species 20–28 of Tryon do not appear closely related to *C. planadae* because of the presence of large, whitish or light brown scales that are concolored or discordantly bicolored. Because of its much smaller, fewer, very dark brown, hardened scales and lack of whitish or hyaline hairs on the blade, *C. planadae* does not seem to be a member of the *C. fulva* (M. Martens & Galeotti) Fée group either (Tryon's spp. 29–34). The presence of spines, light colored petioles, and lack of petiole scurf suggest that *C. planadae* is also not close to spp. 37–40 of Tryon, the group pf *C. lechleri* Mett. Almost by default, this leaves only the *C. caracasana* (Klotzsch) Domin group (including *C. dissoluta* Jenm. of Jamacia), which has similarly dark (but thinner, and much more numerous, and much larger) rhizome scales and also agrees with *C. planadae* in general blade dissection (bipinnate-pinnatifid) and presence of dense petiole scurf. However, the species in the *C. caracasana* group lack decumbent stems (as do all other species of *Cyathea*) and also lack the nearly naked greenish stems of *C. planadae* (unique in the genus, as far as we know), and the shared features may not necessarily be indicative of close phylogenetic relationship. Furthermore, there does not seem to be any close relationship of *C. planadae* to any of the Andean species recently described by Moran (1991).

Within the Cyatheaceae, spore morphology has been a useful phylogenetic character (Conant et al., 1996). Spores of *C. planadae* resemble those of *Trichipteris dombeyi* (Desv.) Barrington, *T. cordata* (Klotzsch) R.M. Tryon, and *T. gibbosa* (Klotzsch) Barrington in having a psilate perine (Gastony, 1979). Distinct pitting along the laesural arms is well developed in a number of species of *Cyathea* (sensu Lellinger, 1987) and within the *Cyathea* clade of Conant et al. (1995). In this feature, *C. planadae* is most like *T. schlimii* (Kuhn) Barrington and *T. villosa (Willd.) R.M. Tryon.* Spores of *C. planadae* also resemble those of *T. hirsuta* (C. Presl) R.M. Tryon and *T. rufa* (Fée) R.M. Tryon (Gastony,

1976) but are distinguished by the lack of well-developed pitting over the rest of the exine surface. A distinct lip also occurs in spores of *C. planadae,* as well as in *T. trichiata* (Maxon) R.M. Tryon and *T. scabriuscula* (Maxon) R.M. Tryon (Gastony, 1979). On the basis of spore morphology alone, *C. planadae* fits best with the *T. gibbosa* or *T. armata* (Sw.) R.M. Tryon subclades within the *Cyathea* clade (of Conant et al., 1996), but this affinity is neither supported nor refuted by other morphological features. The similarity of spores of *C. planadae,* an indusiate species, to those of certain species of *Trichipteris* sensu Tryon (exindusiate), lends further support for including a polyphyletic *Trichipteris* within *Cyathea,* a circumscription advocated by Lellinger (1987).

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Distribution of Tree Ferns (Cyatheaceae) Across the Successional Mosaic in an Andean Cloud Forest, Nariño, Colombia

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ABSTRACT.—Approximately eleven species of tree ferns (Cyatheaceae, *Dicksonia*, and *Lophosoria*) occur in the cloud forest of La Reserva Natural La Planada, Nariño, Colombia. We used 500 m^2 (50×10 m) plots, 10 each in primary forest, secondary forest, and abandoned pasture, to measure the density and species distribution of tree ferns. Abandoned pasture and secondary forest had approximately equal tree fern densities, with density in primary forest significantly lower. Species richness was highest in secondary forest due to the mixture of early and late successional species. Pasture and secondary forest habitats were dominated by *Cyathea caracasana*; higher diversity recorded in abandoned pasture is due to high relative abundances of two subordinate species: *Lophosoria quadripinnata* and *Dicksonia sellowiana*. Primary forest was dominated by a single species, *Cyathea planadae*, with very low relative abundance of the other species recorded. The dominance transition during regeneration appears to begin after approximately 20 years of regrowth, suggesting that the disturbance mosaic and the resulting environmental heterogeneity in Andean forests is important in maintaining species diversity in tree ferns. Two recommendations for conservation emerge: 1) Conservation of tree fern diversity depends on the maintenance of a variety of successional habitats within the forest; 2) Tree ferns are an important component of species that colonize abandoned open sites in Andean forests. Their rapid growth rate and preference for open habitat suggest that some tree fern species may be useful in restoration strategies in cloud forests.

Tree ferns are an important component of the vegetation of mid- and high elevation cloud forests in the Andes of South America. Within these forests, tree ferns occupy a wide variety of habitats, including primary forests (forest that has not been disturbed by human activity), secondary or regenerating forests, and open habitats such as abandoned pasture or farmland. Tree ferns extend from sea level to the tree line at approximately 3,500 m, and individual species show climate-related altitudinal ranges (Tryon, 1976). Despite this important role in Andean forests, there is little detailed information about the distribution of species across the habitat mosaic in these forests. This study asks: What are the patterns of diversity and species relative abundance observed in different successional habitats in a mid-elevation forest? How does the species composition of tree ferns change across the successional mosaic? And what is the role of open habitats, including forest canopy gaps, in the regeneration of tree fern species? Casual observations at La Planada suggest that there are substantial differences in the composition of the tree fern flora across the successional mosaic. This study documents quantitatively the patterns of composition and diversity-dominance across a range of habitats. The

FIG. 1. Location of La Reserva Natural La Planada in Colombia.

project is part of a long-term study directed by N.C. Arens that includes aspects of the demography, growth, and reproduction of *Cyathea caracasana* (Klotzsch) Domin and *Cyathea planadae* N.C. Arens & A.R. Sm., (Arens and Sánchez Baracaldo, 1994; Arens, 1996; Arens and Smith, 1998).

We focused on three habitat types that are common in Andean cloud forests: primary forest undisturbed by human activity; secondary or regenerating forest; and open habitat, which includes pasture and agricultural land that has been abandoned to recolonization by forest species. On the landscape, these habitat types form a mosaic within which plants, including tree ferns, can disperse. Temporally, these habitats may represent a successional gradient that long-lived plants may experience during their lifetimes. However, because the specific pattern of succession in a given patch will be strongly influenced by the species composition of the surrounding forest, we consider our data as representing a snap-shot view of a dynamic landscape mosaic.

STUDY SITE AND METHODS

La Reserva Natural La Planada is located between 1,850 and 2,300 m above sea level on the Pacific slope of the Andean Cordillera in Nariño, Colombia, 1°09′37″N, 77°59′13″W (Fig. 1). La Planada receives about 4,500 mm of rainfall annually (cumulative data from 1982 to 1996), with a "dry" season from June to August. Cloud harvesting approximately doubles the moisture available to plants (C. Ríos, Instituto von Humboldt, Villa de Leyva, Colombia, unpub-

lished data). Average daily temperature is 19°C throughout the year. Natural vegetation within the reserve is typical of Andean cloud forest, with a canopy of approximately 25 m composed of relatively few tree species. The most common canopy trees include *Alchornea* (Euphorbiaceae), *Clusia* (Guttiferae), *Inga* (Fabaceae), *Miconia* (Melastomataceae), *Myrica* and *Psidium* (Myrtaceae), and *Otoba* (Myristicaceae). The majority of the reserve's 3,200 hectares are covered with primary forest, which has been little-disturbed by human activity. Within primary forest, canopy gaps are an important part of the habitat dynamic. Forest turnover due to gap formation and canopy closure is estimated at 3% annually (Samper, 1992). The reserve also contains areas of recently abandoned pasture-land and secondary forest, which represent less than 50 years of natural regeneration. Land use history information available at the reserve allows precise estimation of regeneration-age, and thus makes La Planada a valuable site for studies of successional floristics.

Field work was completed in February and March 1994. We marked 10 plots, 50× 10 m (500 m^2) of random orientation in each of three habitat types: primary forest, secondary forest, and open habitat. Primary forest was defined as forest that has been little-disturbed by human activity and was identified by the presence of large-diameter canopy trees, emergent trees, and trees of many diameter classes. The understory of primary forest is characterized by greater light environmental heterogeneity than secondary forest, and canopy gaps are common. Secondary forest was recognized by land-use history reports of previous clearing and abandonment to forest regeneration. Secondary forest is characterized by lower light levels in the understory and less heterogeneity in light environment than primary forest. Diameter variation among canopy trees is limited as canopy trees represent a single size-age class at this early stage of regeneration. For this study, open habitat was defined as vegetation in which tree ferns form the canopy and thus are exposed to full sun.

In primary forest two areas were chosen for plots: one in flat topography and one that included slopes. Five plots were located in each area. A canopy tree was chosen as the initial corner of each plot. From the corner tree, the long side of the plot was measured along a randomly generated compass direction (1–360°). Subsequent plots were laid in similar fashion, taking care that plots did not overlap. Four areas of secondary forest were located; three plots were located in each of three areas and one plot located in the fourth. Plots were laid using a technique similar to that described above for primary forest. Open habitat plots were located in three abandoned pastures within the reserve. Orientation of open habitat plots was constrained by the shape of the available pasture. In all three areas, plots were alligned sub-parallel to each other to avoid overlapping.

In each plot, we identified and counted all individuals of erect-trunk tree ferns (Cyatheaceae, *Dicksonia,* and *Lophosoria*) (Table 1). In addition to individuals with well-developed trunks, we included all identifiable sporelings. Most sporelings had not yet developed trunks but were identifiable based on scale and frond characters. With these data, we calculated density of individ-

TABLE 1. List of the tree fern species reported in La Reserva Natural La Planada, Nariño, Colombia, including collections made by the late Alwyn Gentry and P. Sánchez Baracaldo, and observations by N.C. Arens.

Family	Species
Cyatheaceae	*Alsophila erinacea* (Hook.) Conant
	Alsophila sp.
	Cnemidaria quitensis (Domin) R.M. Tryon
	Cyathea caracasana (Klotzsch) Domin
	Cyathea multiflora Sm.
	Cyathea planadae N.C. Arens & A.R. Sm.
	Cyathea thrichiata (Maxon) Domin
	Cyathea ulei (H. Christ) Domin
	Sphaeropteris cuatrecasasii R.M. Tryon
Dicksoniaceae	*Dicksonia sellowiana* Hook.
Lophosoriaceae	*Lophosoria quadripinnata* (J. Gmel.) C. Chr.

uals per plot in each habitat and documented the distribution of species across the successional gradient.

Descriptive indices of tree fern diversity within the three successional habitats were calculated using two commonly used metrics: the Simpson diversity index (D) and the Shannon-Wiener diversity index (H) (Begon et al., 1990) (Table 2). Species richness (s) is the number of species encountered in plots within each habitat. The Simpson diversity index (D) calculates diversity within each habitat including both the number of species and the relative abundance (P) of each species (*i*) (Equation 1). Relative abundance (P) is calculated as the number of individuals of species *i* divided by the the total number of individuals of all species.

$$\text{Simpson diversity index} = D = \frac{1}{\sum_{i=1}^{s} P_i^2} \quad (1)$$

Calculation of equitability (E) (Eq. 2) represents the dominance structure among species.

$$\text{Equitability} = E = \frac{1}{\sum_{i=1}^{s} P_i^2} \times \frac{1}{s} \quad (2)$$

For example, when comparing two communities with an equal number of species (s), the community with a single dominant species and the rest represented by few individuals would generate an equitability value lower than a community with all species of equal relative abundance. The Shannon-Wiener index (H) (Equation 3) calculates diversity and equitability (J) (Equation 4) with similar parameters but using the multiplicative term ln P*i* (base 10 or base 2 logarithms can be substituted for natural logarithms) to enhance the influence of community equitability on the resulting index.

TABLE 2. Descriptive indices of tree fern diversity within the three successional habitats included in this study.

	Primary forest	Secondary forest	Open habitat
Species richness	10	11	8
Simpson diversity index	1.66	2.09	2.58
Simpson equitability	0.17	0.19	0.32
Shannon diversity index	0.97	1.04	1.08
Shannon equitability	0.42	0.44	0.52

$$\text{Shannon diversity index} = H = -\sum_{i=1}^{s} P_i \ln P_i \quad (3)$$

$$\text{Equitability} = J = \frac{\sum_{i=1}^{s} P_i \ln P_i}{\ln s} \quad (4)$$

Statistical analyses were performed with SYSTAT 5.0 for Macintosh (Wilkinson, 1989).

RESULTS

In open habitat, the average density of individuals, including many sporelings, was 83 per plot (Fig. 2) with a range of 21 to 179. This range highlights the heterogeneity in distribution of tree fern individuals in open habitat. In all cases, open habitat sites sampled were within 500 m of a patch of intact forest containing tree ferns, so we assume spores were readily available for colonization of open sites. The majority of individuals present in open habitat sites were *Cyathea caracasana* (overall relative abundance 54%) with some representatives of *Lophosoria* (25% relative abundance) and *Dicksonia* (19% relative abundance). However, open habitat plots differed one from another in species dominance; *Lophosoria quadripinnata* (J. Gmel.) C. Chr. dominated one plot (53%) and *Dicksonia sellowiana* Hook. dominated another (97%). Plants in open habitat had large variability in height, which has been used to estimate tree fern age within habitats (Bittner and Breckle, 1995; Seiler, 1981, 1995). Tree ferns in open habitat ranged from individuals with trunks several meters tall to sterile juveniles that had not yet developed erect trunks. Juveniles tended to occur in clumps.

In secondary forest, the density of individuals averaged 83 tree ferns per plot with a range of 61 to 143 (Fig. 2). *Cyathea caracasana* and *C. planadae* were the most common species. Most individuals of *C. caracasana* were approximately one meter in height, and no sporophyte recruitment was observed. In contrast, all individuals of *C. planadae* encountered in secondary forest plots were less than 10 cm in vertical height. Other species present had developed erect trunks, but none were taller than one meter.

In primary forest, the average density of individuals was 50 per plot with a

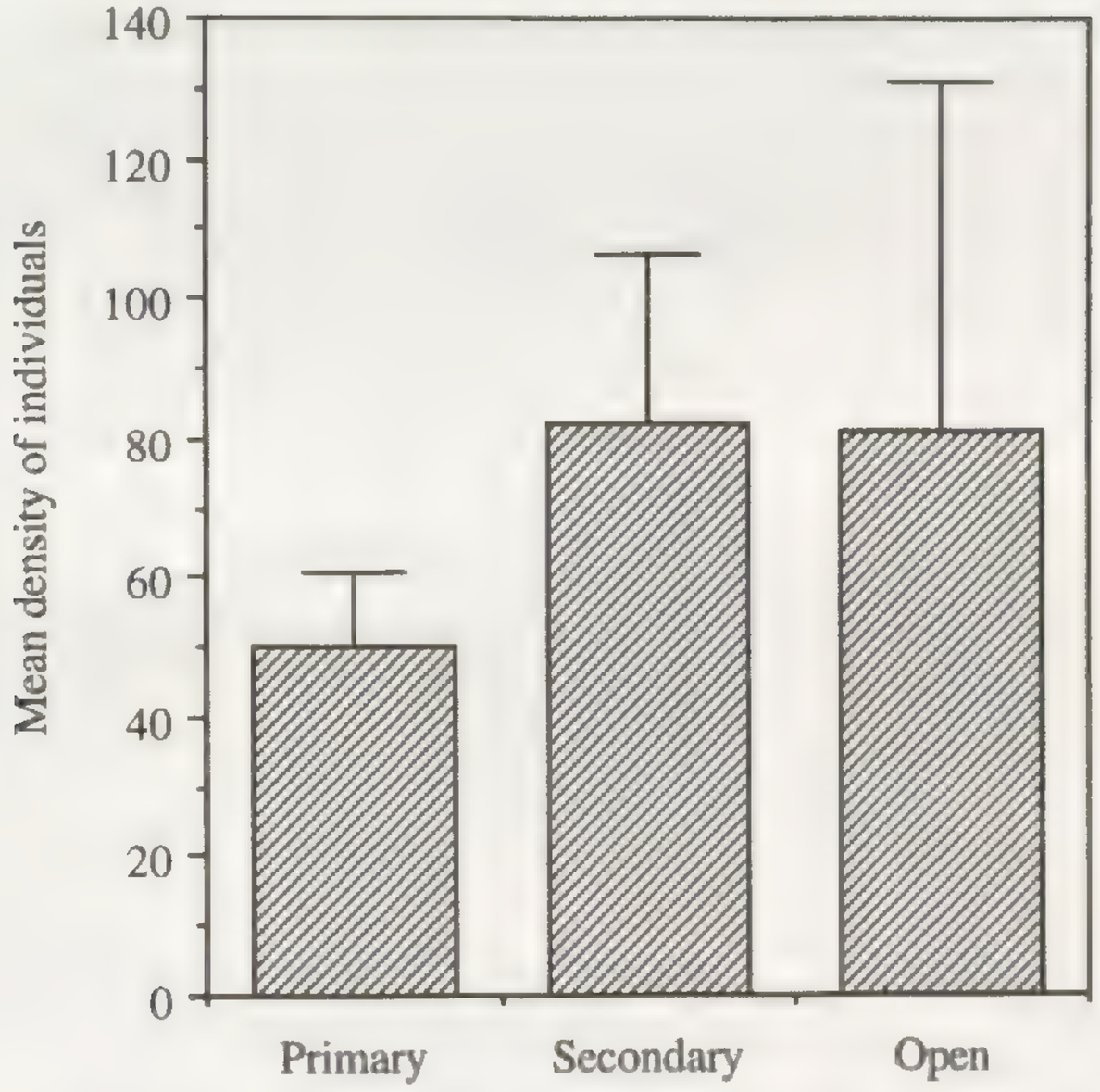

FIG. 2. Average density of individuals per 10 m × 50 m plot in three habitats. Standard errors represent variability between plots in each habitat. Values presented in text are rounded to the nearest whole number.

range of 36 to 66 (Fig. 2). *Cyathea planadae* was the most common species in this habitat and all other species found in primary forest are represented at relative abundances below 10%. Most of the rare species encountered in the field were found in primary forests, and many were represented by only one or two individuals in single plots. This is consistent with the extremely low densities of rare tree fern species observed in the primary forest of New South Wales, Australia (Benson and McDougall, 1993).

At La Planada, sporelings of *C. planadae* were common in the primary forest, but little recruitment was observed in this habitat among species other than *Sphaeropteris cuatrecasasii* R.M. Tryon. A number of the rarest species in primary forest (e.g., *Cyathea multiflora* Sm. and *Cyathea trichiata* (Maxon) Domin) were encountered in groups of four or five individuals, with single taller individuals and several of lesser stature. Many reports conclude that within a given environment, trunk height is a reasonable proxy for age of individual tree ferns (Ash, 1987; Bittner and Breckle, 1995; Seiler, 1981, 1995), whereas great variability in growth rate is observed in individuals of the same species living under different conditions (Arens, 1996; Bittner and Breckle, 1995; Conant, 1976; Tanner, 1983; Tryon and Tryon, 1982). Thus, for a group of co-occurring tree ferns which have lived under the same conditions, trunk height is a reasonable measure of relative age of the individuals. Therefore, it seems likely that the small groups of single rare species reported in primary forest at La Planada are the result of in situ reproduction of a single colonizing individual. For species such as *Cyathea caracasana,* for which we did not observe sporophyte recruitment below a closed canopy, it is possible to con-

TABLE 3. Taxa ranked from most abundant to least abundant in primary forest, secondary forest, and open habitat. Percent relative abundance data for each species in each habitat, rounded in the nearest whole number, are noted in parentheses after each taxon. Relative abundances are calculated as the sum of all plots in each habitat type.

Rank	Primary forest	Secondary forest	Open habitat
1	*Cyathea planadae* (77)	*Cyathea caracasana* (64)	*Cyathea caracasana* (54)
2	*C. caracasana* (8)	*C. planadae* (25)	*Lophosoria quadripinnata* (25)
3	*Alsophila* sp. (3)	*Lophosoria quadripinnata* (2)	*Dicksonia sellowiana* (19)
4	*A. erinacea* (3)	*Alsophila erinacea* (2)	*Alsophila erinacea* (1)
5	*Cyathea trichiata* (2)	*Cyathea ulei* (2)	*C. planadae* (<1)
6	*Sphaeropteris cuatrecasasii* (2)	*Sphaeropteris cuatrecasasii* (1)	*Cnemidaria quitensis* (<1)
7	*Cyathea multiflora* (2)	*Dicksonia sellowiana* (1)	*Sphaeropteris cuatrecasasii* (<1)
8	*Dicksonia sellowiana* (1)	*Alsophila* sp. (1)	*Cyathea ulei* (<1)
9	*Cyathea ulei* (1)	*Cnemidaria quitensis* (1)	
10	*Cnemidaria quitensis* (<1)	*Cyathea trichiata* (<1)	
11		*C. multiflora* (<1)	

clude that the presence of this species in multi-height clusters in primary forest represents reproduction in sunny canopy gaps that have subsequently closed. Canopy turnover is high in La Planada's primary forest and this process may be vital to the maintenance of sun-loving species within the forest.

The density of individuals in open habitat and secondary forest are statistically equal. However, the composition of species in these habitats is different (Table 3). Table 2 shows that regardless of the index used, the diversity of tree ferns is higher in open habitat than in secondary forest. This is due primarily to three taxa with relative abundances greater than 10%: *Cyathea caracasana, Lophosoria* and *Dicksonia* (Table 3). Whereas the number of species (species richness) of secondary forest was greater than that of open habitat, 11 as compared to 8 species, the equitability is higher in open habitat. This reflects the dominance of open habitat by one or two species, and contrasts with closed forest in which more species are represented by fewer individuals each. In primary forest, the density of individuals is lower than that observed in either open or secondary forest habitats ($p = 0.005$). Diversity is also lower in primary forest than in secondary forest or open habitat due to the overwhelming dominance of a single species, *Cyathea planadae* (78% relative abundance).

The rank-abundance diagram (Fig. 3) is a graphical representation of the difference in community equitability between the three habitats studied (Table 3). Open habitats are characterized by a dominant species, *Cyathea caracasana,* with significant representation by *Lophosoria* and some *Dicksonia.* Among our 10 plots, we encountered five additional species that were represented by only a few individuals. The dominance of *C. caracasana* in this habitat suggests that this species (with *Dicksonia* and *Lophosoria*) is important in the initial phases of forest regeneration in this Andean forest. In contrast,

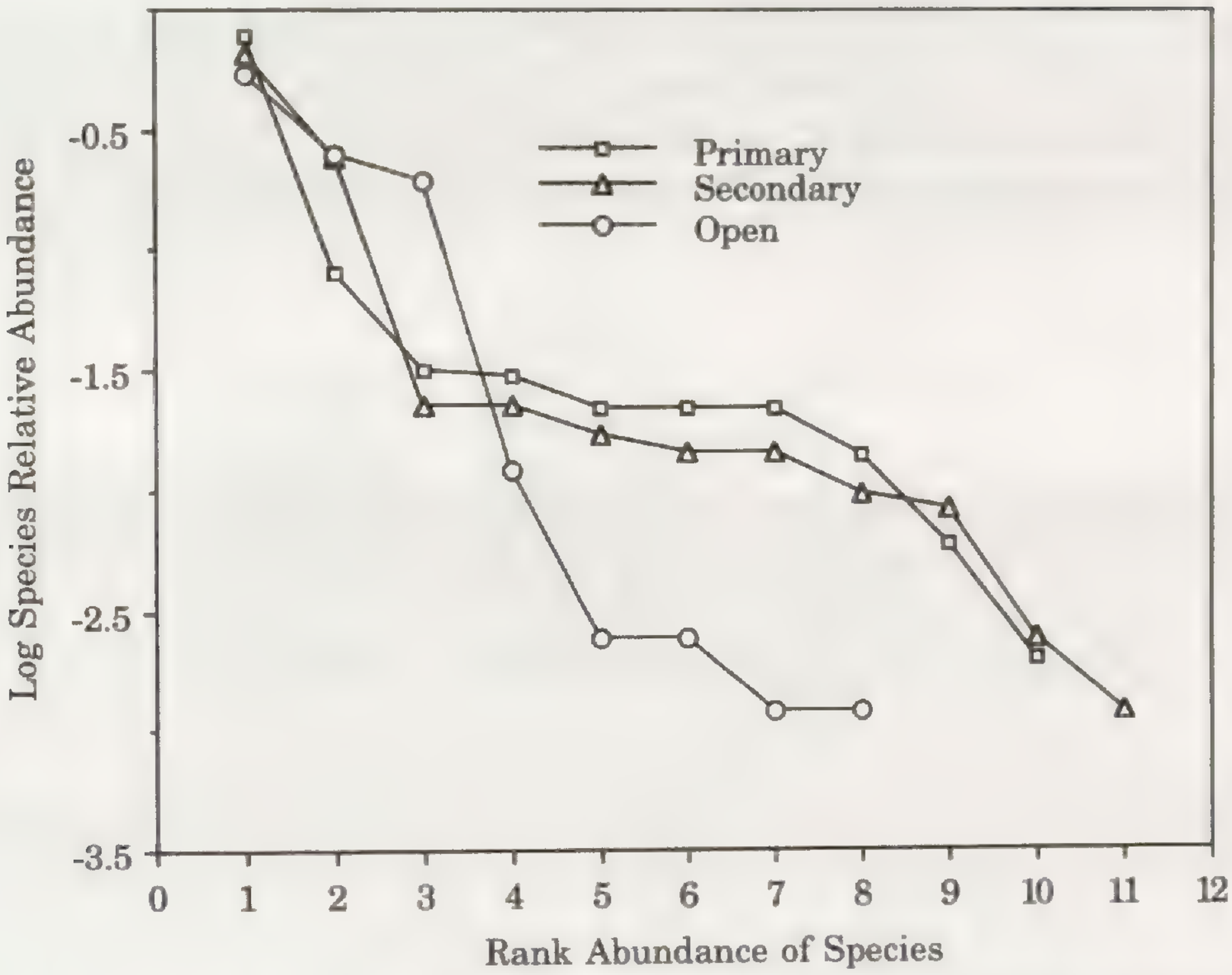

FIG. 3. Rank-abundance diagram for tree ferns in each of three successional habitats. Each line in this diagram is generated by calculating the log of relative abundance for each species found in the habitat. Log values are then ordered (ranked) from largest to smallest and the log value is plotted against rank. The shape of each curve is a graphical expression of the equitability or evenness of the community. Communities with high equitability, in which all species have similar relative abundances, have flat and horizontal curves. Communities dominated by a few very abundant species have strong negative slopes.

the rank-abundance diagrams for mature and secondary forest are very similar, with one or two common species, a few at low abundance, and two or three species at very low abundance. Whereas the rank-abundance diagram makes no distinction between plants of different age classes within a community, we note that all individuals of *C. planadae* observed in secondary forest were sporelings or small plants. This contrasts with primary forest, in which a wide range of size classes of this species were present.

At La Planada, land use records allow us to estimate reliably the length of time each of our secondary forest plots have been regenerating. With this information, we estimate that the transition between a tree fern assemblage dominated by *Cyathea caracasana* and one characterized by *C. planadae* appears to occur after approximately 15 to 20 years of regeneration, when we begin to encounter sporelings of *C. planadae, C. ulei,* and *C. trichiata* in the understory. In plots within stands that have undergone 30 to 40 years of regeneration, the species composition and diversity of tree ferns is very similar to that of primary forest, which may account for the similar rank-abundance profiles (Fig. 3). In older secondary forest, individuals of forest species (e.g., *C. planadae, C. ulei,* and *C. multiflora*) were generally smaller (younger) than individuals in primary forest. Thus, the transition in tree fern diversity and species composition occurs at a relatively early stage of forest succession. It is important

to note that in this study, the spore sources for colonization of open habitat and young secondary forest were nearby. Thus the recruitment of primary forest species can occur almost as soon as the canopy closes and understory microenvironments are suitable for gametophyte establishment.

The Role of *Cyathea caracasana* in the Regeneration of the Cloud Forests of Nariño

Our data from La Planada show that in abandoned pastures, tree fern species such as *Cyathea caracasana* are among the first forest species to establish. In full sun, *C. caracasana* has a growth rate of up to 2 cm per month (Arens, 1996) and can reach a height of up to two meters before woody species form a canopy. Furthermore, individuals of *C. caracasana* in a sunny habitat produce abundant spores and continue recruitment (Arens, 1996).

In contrast, when the canopy closes, *C. caracasana* exhibits a diminished growth rate with little spore production and no recruitment. The species appears to persist in the understory until a canopy gap opens above it to permit further growth and reproduction or the individual dies. In low-canopy secondary forest (canopy less than 3 m height), *C. caracasana* may grow petioles up to 2.5–3 m in length to allow the photosynthetic surfaces of the frond to persist in the canopy. This appears to allow the plant to maintain a growth rate comparable to conspecifics in open habitat. Growth rates calculated for plants in open habitat ("open" in Fig. 4) and those with long petioles in low-canopy forest ("young/long" in Fig. 4) are nearly the same ($p=0.005$), whereas growth rates observed for older, high-canopy forest ("old forest" in Fig. 4) are significantly less (0.1–0.3 cm per month) than those observed in plants experiencing full sun (Arens, 1996). Similarly, growth rates for short-petioled individuals in the younger, low-canopy forest ("young/short" in Fig. 4) are statistically indistinguishable ($p=0.005$) from those of understory plants in older, tall-canopy forest ("old forest" in Fig. 4). Similar patterns are observed in other members of the family. In El Parque Nacional El Ávila in Venezuela, which is characterized by elevation, climate, and vegetation similar to La Planada, Ortega (1984) observed that trunks of *Sphaeropteris senilis* (Kl.) R.M. Tryon [= *Cyathea senilis* (Kl.) Domin] under a closed canopy had a growth rate of 3–4 cm per year. Fronds of *C. senilis* had an average longevity of approximately 216 days (Ortega, 1984), which is almost equal to the average leaf-longevity of *C. caracasana* in closed forest at La Planada (200 days) (Arens, 1996). In contrast, average leaf-longevity of *C. caracasana* in full-sun was 130 days. It is probable that individuals of *Cyathea caracasana* in the understory can persist until a gap forms in the canopy. This change in light environment allows increased growth rate, spore production, and recruitment of sporophytes. This conclusion is consistent with observations of *Cyathea senilis* in Venezuela, which has high light requirements for recruitment, but can persist at low growth rate in the understory (Ortega, 1984). This interpretation is also consistent with the observation of rare individuals of *C. caracasana* in primary

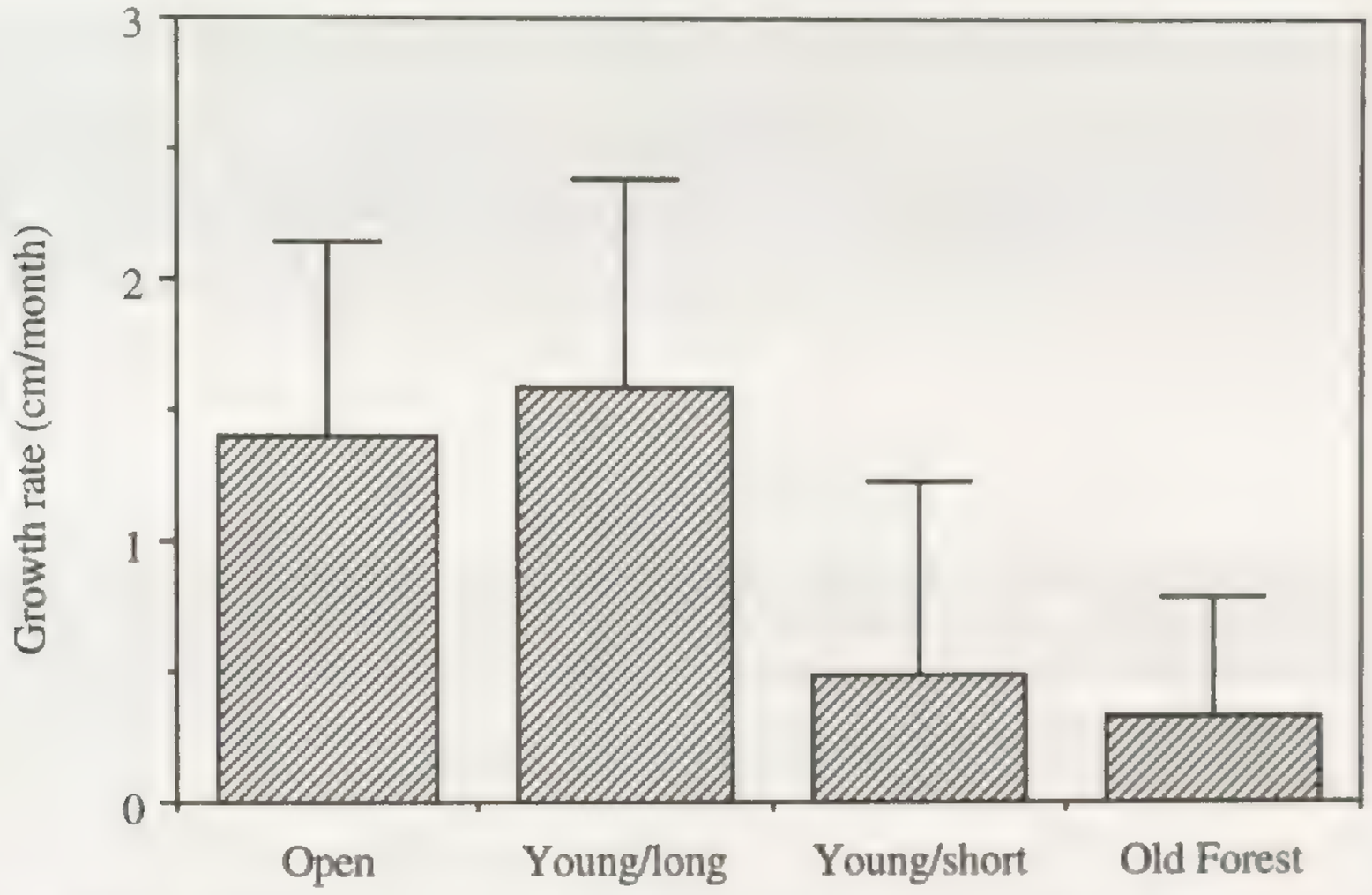

FIG. 4. Growth rate comparison of individuals of *Cyathea caracasana* in full sun (open) habitats, a young, closed canopy forest after approximately 10 years regeneration in which the canopy is only about 3 m in height, and an older secondary forest after approximately 30 years of regeneration in which the canopy is approximately 20 m in height. Sample individuals in the younger forest were divided into two sub-groups: a) Individuals with long petioles (Young/long) (n=11) in which the photosynthetic surface of the frond resides in the canopy, and b) individuals with short (Young/short) petioles (n=9). Growth rates for open habitat and the older secondary forest are calculated based on a sample of 20 individuals. In all cases, growth was observed for 18 months (Arens, 1996).

forest of La Planada, in which the species is found in small groups of multi-height individuals as discussed above.

CONSERVATION OF TREE FERN DIVERSITY

Our data show that tree ferns exhibit a variety of environmental preferences and ecological strategies for coping with the habitat mosaic found in the Andean cloud forest. In fact, this mosaic may be crucial to the maintenance of high species richness in these forests. Two recommendations for the conservation of this group emerge from this study.

1) To maintain tree fern species richness in Andean forests, it is essential to maintain a mosaic of successional habitats. In most cases, human activity near or within protected areas helps to maintain a diversity of open and closed habitats, important to the maintenance of diversity. However, large open areas may present a barrier to colonization by many primary forest taxa. Also, because many primary forest species are rare, it is important to maintain large areas (greater than 100 hectares) of primary forest that can serve as refuges for these taxa. Because rare forest species recruit into regenerating forest and may require many years to reach spore-producing age, the preservation of primary forest refuges is essential. Pangtay et al. (1989) concludes that some species of rare, understory tree ferns such as *Cyathea spinulosa* Wallich ex Hook. are in danger of extinction because of extensive clear-cutting in the Himalayas.

2) Tree ferns (particularly *Cyathea caracasana, Dicksonia,* and *Lophosoria*) are an important part of the vegetation that colonizes open areas and abandoned pastureland in the Andes. As part of the natural cycle of regeneration in cloud forests, tree ferns may be useful partners in restoration of Andean cloud forests. However, further research will be required to establish a strategy for their use.

Acknowledgments

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APPENDIX. Number of stems per plot of eleven tree fern species; ten plots each in three habitat types. Loph = *Lophosoria quadripinnata*; C. car = *Cyathea caracasana*; C. plan = *Cyathea planadae*; C. tri = *Cyathea trichiata*; Sph = *Sphaeropteris cuatrecasasii*; C. ul = *Cyathea ulei*; A. eri = *Alsophila erinacea*; Dick = *Dicksonia sellowiana*; C. quit = *Cnemidaria quitensis*; C. multi = *Cyathea multiflora*; Als = *Alsophila* sp.

Habitat	Plot number	Loph	C. car	C. plan	C. tri	Sph	C. ul	A. eri	Dick	C. quit	C. multi	Als	Total # individuals	Total # species
Primary	1	0	7	36	0	4	0	0	3	0	0	0	50	4
Primary	2	0	8	40	0	0	1	0	0	0	0	0	49	3
Primary	3	0	9	32	3	2	0	5	0	0	0	0	51	5
Primary	4	0	1	16	7	5	0	4	1	0	0	0	34	6
Primary	5	0	0	48	1	0	0	0	0	0	0	6	55	3
Primary	6	0	2	37	0	0	1	0	0	0	2	0	42	4
Primary	7	0	5	52	0	0	0	2	0	0	2	1	62	5
Primary	8	0	5	48	0	0	1	2	1	0	1	8	66	7
Primary	9	0	2	41	0	0	0	2	1	1	6	1	54	7
Primary	10	0	1	34	0	0	0	0	1	0	0	0	36	3
Secondary	1	0	60	25	0	2	2	0	1	3	0	0	93	6
Secondary	2	5	57	4	0	2	8	4	0	0	0	0	80	6
Secondary	3	2	128	8	0	1	0	0	3	1	0	0	143	6
Secondary	4	3	65	8	0	3	0	0	0	0	1	0	80	5
Secondary	5	1	59	4	2	1	0	0	0	2	0	4	73	7
Secondary	6	4	51	4	0	0	0	2	0	0	0	0	61	4
Secondary	7	2	25	27	0	0	0	1	6	0	0	2	63	6
Secondary	8	1	19	33	0	0	2	8	1	0	0	0	64	6
Secondary	9	1	30	38	0	1	1	3	0	1	0	2	77	8
Secondary	10	0	43	49	0	2	1	1	1	0	0	0	97	6
Open	1	8	36	0	0	0	0	0	10	0	0	0	54	3
Open	2	40	72	0	0	0	0	1	9	1	0	0	123	5
Open	3	60	42	0	0	0	0	5	4	1	0	0	112	5
Open	4	20	78	0	0	0	0	1	6	0	0	0	105	4
Open	5	61	112	2	0	1	0	0	3	0	0	0	179	5
Open	6	4	13	0	0	0	0	0	17	0	0	0	34	3
Open	7	0	2	0	0	0	0	0	64	0	0	0	66	2
Open	8	3	9	0	0	0	0	0	38	0	0	0	50	3
Open	9	6	9	0	0	0	0	0	6	0	0	0	21	3
Open	10	7	71	0	0	0	1	3	3	0	0	0	85	5

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The Effect of Sterilization and Storage Conditions on the Viability of the Spores of *Cyathea delgadii*

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ABSTRACT.—Tests were conducted to evaluate the effects of various sterilization procedures on spores of *Cyathea delgadii* stored under different conditions with the intent of developing a strategy of ex-situ spore conservation of this declining species. Although the percentage of germination after two years of storage was relatively low for all storage and sterilization parameters, dry storage at −12°C resulted in the best survival rate. Elimination of bacterial and fungal contaminants was best when the spores were washed, treated with calcium hypochlorite, incubated, filtered, and washed again before sowing on medium containing Nystatin.

The spores of some tree ferns in the Cyatheaceae lose their viability after a few weeks of storage (Page, 1979). The viabilility of *Cyathea delgadii* Sternb. spores is already decreased after only two months storage at 12°C and low humidity (Randi and Felippe, 1988a) and percent spore germination is significantly decreased after six months of storage under the same conditions (Marcondes-Ferreira and Felippe, 1984). In general, it is advisable to store fern spores at low temperature and low humidity, sometimes with the use of desiccators (Dyer, 1979a). More recently, storage in darkness at room temperature and high humidity (imbibed) has been advised for some species (Lindsay et al., 1991).

Before storing imbibed spores in the laboratory or before germination of spores stored dry, there is the need to sterilize them. Several chemicals have been used for this purpose (Dyer, 1979b), and either sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl_2)\cdot 4H_2O$) are the most commonly used. Unfortunately, most treatments that kill contaminant fungi and bacteria also reduce fern spore germination. Different concentrations of chemicals and duration of application have been used. Before germination, it is advisable to incubate the spores in darkness for a period of time: the fungi and bacteria start to grow and become more sensitive to the chemicals used for sterilization (Voeller, 1964; Schedlbauer, 1976).

The objective of this investigation was to study different sterilization methods and storage conditions in order to develop a procedure for maintaining a source of viable spores of *Cyathea delgadii* for "ex situ" conservation, because this species is rapidly disappearing from the Brazilian forests as consequence of commercial exploitation.

Methods and Materials

Fertile leaves were collected from a population of *Cyathea delgadii* Sternb. growing in a gallery forest in the Reserva Biológica e Estação Experimental de Moji Guaçu, São Paulo State, Brazil (22°18′S and 47°11′W). After drying the leaves over absorbent paper at 25°C, the spores were separated from debris and stored in different conditions.

Sterilization Procedures.—For the experiments, the material used (glassware, water, solutions, etc) was autoclaved for 40 min (121°C and 1.2 atm). For experiments on sterilization, four sterilization procedures were tested on spores stored dry at 4°C in darkness.

A1) Successive washings (5, 10, and 15 times) in sterile water, with or without preliminary treatment in sodium hypochlorite. 50mg of spores were placed in centrifuge tubes for 2min in either 0.1% sodium hypochlorite or sterile water; after centrifugation, 2ml of sterile water were added; after 2min, the mixture was centrifuged at 1250× g for 5 min. The supernatant was removed and the washing repeated 5, 10, or 15 times.

A2) Treatment with sodium hypochlorite or calcium hypochlorite at 0.5%, 1.0%, 3.0%, and 5.0%. 50 mg of spores were kept in the different solutions for 7 min; after centrifugation they were washed 5 times in sterile water.

A3) After preliminary treatment in calcium hypochlorite, Nystatin (Squibb, 100U/ml^{-1}) was added to the agar after autoclaving and cooling. The spores were treated previously with 0.5% calcium hypochlorite for 2 min and washed 5 times in sterile water.

A4) Pre-incubation (incubation before sowing) in darkness for 5 days followed by filtration.

Washed spores (5 times, in sterile water) or non-washed spores were subjected to pre-incubation with or without washing. Washed or non-washed spores were incubated in sterile water in darkness at 25°C for 5 days. They were treated with a soap solution "Tween 20" (100µl of a solution prepared with 2 drops of Tween in 100ml of distilled water) to kill metabolically active cells. To separate fungal hyphae, the mixture was filtered through a nylon mesh filter with 64µm diameter pores (Lockertex N64, Warrington, Cheshire, UK) which retained the hyphae. *Cyathea delgadii* spores, which require light for germination and thus remained dormant, were retained on a second filter with 20µm diameter pores (Lockertex N20). The filters were immersed in 0.5% sodium hypochlorite for 30 seconds and then washed in sterile water.

Storage Conditions.—Freshly collected spores were stored as follows.

B1) in sealed glass bottles (with plastic caps) in darkness for up to 24 months at 4°C or at 25°C.

B2) in glass bottles covered with gauze, kept in a desiccator (silica gel) in darkness for 24 months at 4°C or at 25°C

B3) in sealed transparent square (3×3cm) plastic bags kept in darkness at 25°C and −12°C for 24 months

TABLE 1. Effect of 0.1% sodium hypochlorite (NaOCl) for 2 min and successive washings with sterile water on contamination by bacteria and fungi of spores of *C. delgadii.* Spores incubated for 7 days in water at 25°C before examination.

Treatment	Number of washings	% of spots	
		Bacteria	Fungi
No NaOCl	5	89.3	5.0
	10	100.0	5.0
	15	100.0	1.7
With NaOCl	5	20.0	85.0
	10	16.7	95.0
	15	36.7	76.7

B4) on 1.2% agar (in water) in sealed transparent square (3×3cm) plastic bags kept in darkness at 4°C or 25°C for 24 months.

In addition, spores previously stored in glass bottles in darkness at 4°C for four months were sown in Petri dishes (5cm diameter) on 15ml of 1.2% agar (in mineral nutrient solution or water). To keep the spores wet but not in direct contact with the agar during storage, disks of different materials (all Millipore filters) were placed over the agar before sowing. In this way, a large amount of spores, as would be required for biochemical determination, could be kept free of contamination with agar. The treatments were as follows: a) spores sown direct onto agar (control); b) spores sown onto a disk (4.5cm diameter) of fibreglass placed on top of the agar; c) spores sown onto a disk (3.8 cm diameter) of cellulose placed on top of the agar; d) spores sown onto a disk (4.5cm diameter) of nylon placed on top of the agar; e) spores sown onto a disk (4.5cm diameter) of acetate placed on top of the agar.

On each disk were sown 100mg of spores and a few drops of water (to wet the material). The closed Petri dishes were maintained upside down at 25°C in darkness for two weeks storage. Three Petri dishes were used in each treatment. For the germination tests the Petri dishes were transferred to white light at 25°C.

GERMINATION.—Germination procedures were described fully in Esteves and Felippe (1985). Germination was tested in water or Mohr's nutrient solution (liquid medium), as presented by Dyer (1979b), under continuous white fluorescent light (320μW.cm $^{-2}$) at 25°C. Germination was carried out in conical flasks (3 flasks per treatment and 15mg of spores per flask). Two slides were prepared for each flask and a total of 100 spores was counted in each slide. Germination was recorded as the emergence of the rhizoid. According to Randi and Felippe (1988b), maximum percent germination in this species is reached by day 7 after sowing spores stored up to 190 days. Thus, in the present paper, germination is always recorded on day 7 from the moment of sowing the spores. It is therefore possible to detect if germination is unnaffected, which is the objective, but it should be noted that a reduced germination percentage at day 7 could be a result of either delayed germination or loss of viability.

TABLE 2. Effect of 7 min in sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl_2)$) on the contamination by fungi of spores of *C. delgadii* (stored dry for four months in darkness at 4°C) during germination on 1.2% agar (in mineral nutrient solution) at 25°C under continuous white light; results on day 7 from the sowing of spores on agar. Lowercase letters compare values within and between columns and rows.

Hypochlorite concentration	Contamination % of spots				*C. delgadii* germination %	
	$Ca(OCl_2)$		NaOCl			
%	Fungal	Bacterial	Fungal	Bacterial	$Ca(OCl_2)$	NaOCl
0.0	100.0	100	100.0	100	57.8a	57.8a
0.5	100.0	0	100.0	0	52.3a	51.0a
1.0	100.0	0	78.3	0	52.8a	38.2b
3.0	98.0	0	40.0	0	48.8a	3.2c
5.0	96.7	0	3.3	0	58.2a	0.0c

When the effect of storage on germination was studied, Nystatin was added (100U/ml^{-1} of germination medium).

Germination also was tested on solid medium. In this case, 1.2% agar was used, prepared with either water or mineral nutrient solution and placed in 5 cm Petri dishes (two Petri dishes were used per treatment). In each Petri dish were placed 10 spots (4 mm diameter) of spores. The dishes were incubated at 25°C under white light. In each spot, 100 spores were counted under a stereomicroscope (20 separate counts per treatment). A detailed description of the procedures are in Simabukuro (1995). As germination was delayed in solid medium (Simabukuro, 1995), germination was measured on days 7 and 14.

Germination results (in percentage after arc sine transformation), were subjected either to the "Student's T test" or to analysis of variance and $LSD_{5\%}$ (Tukey) according to Snedecor (1962). In Tables 2–6, the results of the statistical analyses are represented by lowercase letters (the same letter means acceptance of the null hypothesis). The comparison is either within the column or within the row.

RESULTS AND DISCUSSION

STERILIZATION.—Contaminants like bacteria and fungi do not interfere with spore viability during dry storage, although their presence may be a disadvantage during subsequent germination and development. However, during wet storage, the viability of fern spores can be strongly affected by contaminants. Several sterilization procedures were tested in conjunction with wet storage. The procedures included some that involved little or no chemical treatment in order to minimize adverse effects of sterilants on fern spore viability.

Schwabe (1951) used washing in sterile water to sterilize spores of *Pteridium aquilinum*. Warne et al. (1986) advised washing the spores with sterile water together with sodium hypochlorite. The effects of successive washings and sodium hypochlorite (see Methods and Materials, A1) on growth of contami-

TABLE 3. Effect of Nystatin added to 1.2% agar (mineral nutrient solution) on contamination by bacteria and fungi of spores of *C. delgadii* (stored dry for four months under darkness at 4°C) during germination at 25°C under continuous white light; results measured on day 7 from the sowing of spores on agar. Before the germination test the spores were treated with 0.5% calcium hypochlorite for 2 min and washed five times with sterile water. Lowercase letters compare values within each column.

	Contamination % of spots		*C. delgadii* germination
Treatment	Fungal	Bacterial	%
No Nystatin	86.0a	100.0a	57.8a
With Nystatin	18.3b	48.3b	52.7a

nants (fungi and bacteria) during imbibition (in water) of spores of *C. delgadii* can be seen in Table 1. Increased number of washings had no effect on reducing bacterial infection, but this was strongly reduced when the spores were pretreated with 0.1% sodium hypochlorite. Fungal infection was very low after washing just with sterile water, but was strongly increased by pretreatment with 0.1% sodium hypochlorite.

Pretreatment with sodium hypoclorite in concentrations above 0.5% reduced fungal contamination, but the germination at day 7 (on 1.2% agar) of spores of *C. delgadii* also was reduced (Table 2). Fungal contamination was not reduced by pretreatment with calcium hypochlorite at concentrations up to 5% (see Materials and Methods, A2); this substance also had no effect on the germination of *C. delgadii* spores. Both chemicals strongly reduced bacterial contamination (Table 2) and have been used with success by several workers in different combinations of concentration and time of application. Thus, sodium hypoclorite has been used at 0.5% for 25 seconds for *Pteridium aquilinum* or 15% for 10 min for *Anemia phyllitidis,* and calcium hypoclorite at 1% for 4 min for *Ceratopteris thalictroides* or at 15% for a very short period (time not measured) in *Thelypteris palustris* (Bell, 1958; Näf, 1958; Weinberg and Voeller, 1969; Schedlbauer, 1976).

Nystatin in the agar medium (see Materials and Methods, A3) strongly reduced fungal contamination (and also reduced bacterial infection) while not affecting the germination of the fern spores (Table 3). Nystatin is widely used to reduce contamination by fungi during germination of seeds and has no effect on gametophyte development (Dyer, 1983).

Bacterial contamination was strongly reduced when the spores were washed before and after the pre-incubation and filtration treatments (see Materilas and Methods, A4); no fungal contamination occurred. In Table 4, it can be seen that this sterilization treatment did not affect the germination of *Cyathea* (for storage of spores see Materials and Methods, B1). Pre-incubation/filtration also was successful with spores of *Anemia phyllitidis, Ceratopteris richardii, C. thalictroides, Lygodium,* and *Pteridium aquilinum* (Voeller, 1964; Schedlbauer, 1976).

DRY STORAGE.—After dry storage, the spores were sterilized with 0.5% calcium hypochlorite for 2 min before the germination tests and Nystatin was used in

TABLE 4. Effect of pre-incubation and of washing and filtration before and after pre-incubation upon contamination from bacteria in 12 months old spores of *C. delgadii* (stored dry in darkness at 4°C) during germination on 1.2% agar (mineral nutrient solution) at 25°C under continuous white light. Results on day 7 from the sowing of the spores on agar. Fungal contamination was zero in all treatments. Lowercase letters compare values within each column.

Treatment	Bacterial contamination %	*C. delgadii* germination %
Pre-incubation only + filtration	100.0	31.5a
Pre-incubation + washing + filtration	83.3	23.8a
Washing + pre-incubation + filtration	52.0	30.7a
Washing + pre-incubation + washing + filtration	21.7	35.7a

the germination medium to reduce bacterial and fungal contamination (Tables 2, 3).

Germination on day 7 (from sowing) of spores stored for two months in glass bottles closed with gauze, in darkness (see Materials and Methods, B2) was statistically higher after storage at 4°C than at 25°C (Table 5). When stored at 4°C, germination was 24.7% after 12 months and 1.8% after 24 months. There was no germination after storage at 25°C for 12 or 24 months. However, when spores werestored for 12 months in glass bottles (covered with gauze) in darkness in a desiccator, the effect of 4°C and 25°C on germination was the same. Thus, during the first year there was no effect of temperature during storage in very dry conditions. The effect of temperature was evident after 24 months storage. Thus, from these data it can be concluded that storage at 4°C is better when the spores are kept in dryer conditions (desiccator) for periods longer than 12 months.

The germination of spores stored in sealed small plastic bags (see Materials and Methods, B3) at 25°C and −12°C (deep-freeze) can be seen in Table 6. Germination was very high when spores were kept at −12°C in sealed bags for 12 months. Even after 24 months storage at this temperature, 18.5% of the spores germinated. It is interesting that such a low temperature is tolerated by a species that rarely experiences temperatures as low as freezing (in the Atlantic forest in São Paulo temperature reaches −2°C for a few days during July).

STORAGE IN WET MEDIUM.—Storage at room temperature and high humidity in darkness has been successful in maintaining the viability of spores in several species, such as *Athyrium filix-femina, Blechnum spicant, Equisetum hyemale, Asplenium scolopendriu., Polystichum setiferum, Psilotum* sp., and *Todea barbara* (Lloyd and Klekowski, 1970; Whittier, 1990; Lindsay et al., 1991). Storage in water vapor increased the sensitivity to light of spores of *Cyathea delgadii* (Randi and Felippe, 1988a). Because of these data, we decided to test the effect of wet storage on *Cyathea delgadii* viability.

Spores were kept for 24 months (see Materials and Methiods, B4) mixed with 1.2% agar in sealed small plastic bags at 4°C or 25°C. At 25°C, subsequent germination at day 7 was 12.2% and at 4°C germination was 3.6%. These

TABLE 5. Viability of spores of *C. delgadii* stored in glass bottles closed with gauze at 4°C or 25°C under darkness with or without a desiccator. Germination in liquid medium (mineral nutrient solution) at 25°C under continuous white light. Results on day 7 from the sowing of the spores. Values indicated with a dash (—) were not determined. Lowercase letters compare values within each row.

Period of storage months	Germination %			
	In desiccator		No desiccator	
	25°C	12°C	25°C	12°C
2	—	—	73.8a	80.7b
12	45.0a	41.5a	0.0	24.7
24	8.0b	19.3a	0.0	1.8

results are different from the ones using spores stored in dry condition (Table 5) in which germination was higher at 12°C than at 25°C. It is possible that anaerobic conditions in the sealed bags had an effect on the aging process. It is significant to note that germination after wet storage for 24 months at 25°C in plastic bags was greater than after dry storage in plastic bags.

It is very troublesome to remove spores from the agar (and to have clean spores) when biochemical tests are required. For this reason, tests were done in which spores were placed on disks over agar (and thus not in contact with the agar) for a short storage period (two weeks) to see if the disks would affect *C. delgadii* germination.

The spores of *C. delgadii* (previously stored dry) were stored wet for an additional two weeks on 1.2% agar (prepared with water or nutrient solution) and with or without disks of fibreglass, cellulose, nylon and acetate (see Materials and Methods). Germination in this case was recorded on days 7 and 14 (as germination was delayed on solid medium, according to Simabukuro [1995]).

The results of the germination experiments can be seen in Table 7. On day 7 from incubation in light, there was a reduction of germination with the use of fibreglass, cellulose, nylon, and a strong reduction when acetate was used. By day 14, the use of acetate strongly reduced germination when water (in agar) was used as medium; there was no difference in all the other treatments

TABLE 6. Viability of spores of *C. delgadii* stored in sealed square (3 × 3 cm) plastic bags at −12°C and 25°C in darkness for 24 months. Germination in liquid medium (mineral nutrient solution) at 25°C under continuous white light. Results on day 7 from the sowing of the spores (spores stored dry in darkness at 4°C). Lowercase letters compare values within each row.

Period of storage months	Germination %	
	25°C	−12°C
3	8.0a	66.8b
12	0.0	74.0
24	0.0	18.5

TABLE 7. Germination of spores of *C. delgadii* stored for two weeks on 1.2% agar (water or mineral nutrient solution) covered with or without a disk of fibreglass, cellulose, nylon, or acetate at 25°C in darkness. For germination, the Petri dishes were transferred to continuous light at 25°C. At the time of sowing on agar, the spores had been stored dry for four months in darkness at 4°C.

		Germination %	
Disk	1.2% agar	Day 7	Day 14
Control (no disk)	water	48.0	53.0
	nutrient solution	50.5	55.5
Fibreglass	water	30.0	51.5
	nutrient solution	32.0	58.5
Cellulose	water	16.0	53.0
	nutrient solution	22.0	62.0
Nylon	water	27.0	48.0
	nutrient solution	22.5	54.5
Acetate	water	2.0	22.5
	nutrient solution	0.5	49.5

in relation to germination. However, in the treatments without the use of disks (control) there was contamination by bacteria. In addition to causing the lowest reduction in germination at day 7, fibreglass was the best disk type used because it remained wet for a longer period of time, and was the best substrate for gametophyte development as the rhizoids could grow in between its fibres. The effect of these conditions on viability of spores after long term (12 or 24 months) storage are not known. No differences in germination values could be seen between the use of water or mineral nutrient solution to prepare the agar except on day 14, in the test when acetate was used.

CONCLUSIONS

When wet storage is required, bacterial and fungal contamination is likely to be most effectively reduced when the spores are washed, treated with calcium hypochlorite, incubated, filtered, and washed again before sowing on medium containing Nystatin. This sterilization could also be used after dry storage. However, this combination was not tested by us.

In the case of *C. delgadii,* germination was low after 24 months storage in both dry and wet conditions, i.e., viability is reduced independently of storage conditions in 24 month old spores. The best results were obtained with sealed plastic bags (air dry storage) at −12°C or in gauze-stoppered glass bottles in a desiccator at 4°C. This indicates that a desiccator at −12°C might give better results. It is also possible that storage of imbibed spores in the dark in aerobic condition on the surface of a suitable moist substrate (as in Lindsay et al., 1991) will maintain higher levels of viability. It is worth mentioning that the viability of spores of the tree fern *Cyathea spinulosa* was maintained after 3 days storage in liquid nitrogen (Agrawal *et al.,* 1993); in a preliminary exper-

iment, the viability of spores of *C. delgadii* also was maintained after 12 hours storage in liquid nitrogen.

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The Effect of Surface Sterilization on Cultures of *Ceratopteris richardii* Gametophytes

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ABSTRACT.—Gametophytes of *Ceratopteris richardii* were grown as single-spore and double spore cultures on agar to test the effects of surface sterilization of spores (with bleach) and contamination of cultures on growth and sexual expression. Hermaphroditic gametophytes were larger in double-spore than in single-spore cultures for cultures derived from both untreated and sterilized spores, whereas gametophytes were larger in single-spore than in double-spore cultures in contaminated cultures. However, gametophytes did not differ in size between double-spore and single-spore cultures derived from surface sterilized spores. The greatest antheridiogen response was observed in cultures with no treatment and the least antheridiogen response was observed in contaminated cultures.

A standard method of preparing gametophyte cultures from spores involves the surface sterilization of spores using a dilute bleach (hypochlorite) solution (e.g., Banks et al., 1993). The purpose of this method is to reduce the effects of other organisms on treatments during experimental investigations of gametophyte growth and development. We, however, have been concerned that bleach may affect the extracellular matrix of the spore. An effect on the extracellular matrix of the spore could significantly affect investigations of aspects of gametophyte development such as antheridiogens, as antheridiogens must first affect the extracellular matrix of a spore or gametophyte cell to have an effect on spore germination and gametophyte development. To address the effects of contamination and surface sterilization of spores, we cultured gametophytes of the leptosporangiate fern *Ceratopteris richardii* Brongn. using untreated and surface sterlized spores, culturing untreated spores in both axenic and contaminated media.

MATERIALS AND METHODS

Spores (Hn-n) of *C. richardii* were provided by Thomas Warne of the University of Tennessee at Knoxville. All spores were imbibed in the dark for two days at 10°C in autoclaved deionozed filtered water. To investigate the effects of surface sterilization, spores were surface sterilized prior to imbibition by sprinkling them onto a 0.45μm Millipore filter and washing them with commercial bleach diluted 1:5 with autoclaved deionized filtered water. The filter containing the spores was then washed five times with autoclaved deionized filtered water. All spores were sown onto a nutrient agar medium in 20mm diameter plastic culture dishes as described by Klekowski (1969), with the

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exception that 1.5% agar was used to reduce evaporation. Spores were sown using an Eppendorf pipettor as described by Sayers and Hamilton (1995). Single spore and double spore cultures were prepared as described by Sayers and Hamilton (1995). Twenty-five single spore (SS) and 25 double spore (DS) cultures were prepared from surface sterilized spores, and 50 single spore and 50 double spore cultures were prepared from untreated spores. Twenty-five untreated single spore and 25 untreated double spore cultures were contaminated by pipetting 50μl of a 50ml soil/50ml tapwater solution directly onto spores previously sown onto the nutrient agar medium. There were thus 25 double and 25 single spore cultures for each of three treatments, surface sterilized (labeled "Bleach" in data analysis), contaminated, and untreated. Cultures were placed on a light bench with continuous incandescent and fluorescent light for 14 days at temperatures ranging from 24–26°C. Cultures were enclosed within a canopy of plastic wrap to reduce evaporation.

Gametophytes were harvested and placed in a drop of water on a microscope slide. Size and gender were observed using a Wild microscope at 40× magnification. Size was recorded as the length and width of a gametophyte, with those dimensions used to calculate an ellipse as an estimate of gametophyte size. Gender was observed as: hermaphrodite (H), for gametophytes having both antheridia and archegonia as well as a notch meristem; classic small male (cM), for gametophytes with a large number of antheridia and lacking a notch meristem; notched male (nM), for gametophytes with a few antheridia and having a notch meristem; female (F), for gametophytes having archegonia and a notch meristem; and asexual (A), for gametophytes lacking gametangia and a notch meristem. An antheridiogen response was defined as the presence of cM gametophytes in cultures. Notched male and F gametophytes were considered as immature H gametophytes, and thus classified as H gametophytes for data analysis.

Size data were analyzed using the one-way analysis of variance routine in *PC-Statistics* (Marvasti, 1991). Treatment means were compared using the least significant difference test, as described by Steele and Torrie (1980).

Results

There was no contamination observed in either the cultures derived from surface sterilized spores or untreated cultures, but substantial contamination was observed in contaminated cultures. Fewer than 25 of all SS cultures consisted of a single gametophyte, and fewer than 25 of all DS cultures consisted of a pair of gametophytes (Table 1), due mostly to a failure of spore germination.

There were no cM gametophytes in single spore cultures. In double spore cultures, cM gametophytes appeared in 5 of 17 (29.4%) contaminated cultures, 8 of 18 (44.4%) cultures derived from surface sterilized spores, and 13 of 20 (65%) cultures derived from unsterilized and uncontaminated spores (Table 1). There was significant variation in the size of H gametophytes among treatments ($F=7.36$, $df=5, 138$). The largest H gametophytes were derived from SS

TABLE 1. Gametophyte size and gender. SS indicates gametophytes from single spore cultures. DS indicates gametophytes from double spore cultures. Sizes in mm^2. H includes all hermaphrodites, notched males and females, M indicates classic small males, and A indicates asexual gametophytes.

	Contaminated		Untreated		Bleach	
	SS	DS	SS	DS	SS	DS
1.	1.21 H	4.04 H	0.84 H	1.91 H	1.33 H	1.03 H
		4.67 H		0.68 H		0.03 M
2.	1.27 H	0.07 M	1.68 H	1.37 H	1.07 H	0.46 H
		0.01 A		1.64 H		0.63 H
3.	4.26 H	2.90 H	0.51 H	1.22 H	1.46 H	1.50 H
		1.70 H		1.17 H		0.85 H
4.	1.00 H	1.37 H	0.37 H	0.37 H	1.49 H	0.63 H
		2.64 H		0.10 M		0.46 H
5.	5.67 H	0.91 H	0.32 H	0.46 H	1.22 H	0.07 M
		0.06 M		0.43 H		0.91 H
6.	1.20 H	2.60 H	0.38 H	0.07 M	1.00 H	0.53 H
		0.32 H		1.84 H		0.50 H
7.	1.37 H	0.81 H	0.31 H	1.18 H	1.07 H	0.37 H
		0.95 H		1.94 H		0.37 H
8.	4.26 H	0.27 H	0.48 H	1.00 H	0.81 H	0.05 M
		0.09 M		0.95 H		0.77 H
9.	1.7 H	0.49 H	1.10 H	0.11 H	1.17 H	0.53 H
		0.49 H		1.17 H		0.09 M
10.	1.07 H	0.18 H	0.31 H	0.31 H	1.07 H	0.05 M
		0.49 H		0.05 M		0.72 H
11.	2.91 H	1.21 H	0.68 H	1.70 H	1.46 H	0.37 H
		1.00 H		0.10 M		0.49 H
12.	1.46 H	0.72 H	0.37 H	0.05 M	0.80 H	0.63 H
		0.97 H		2.90 H		0.69 H
13.	1.37 H	0.37 M	0.13 H	0.09 M	0.80 H	0.37 H
		0.92 H		0.27 H		0.37 H
14.	0.63 H	1.84 H	0.59 H	6.83 H	0.36 H	0.05 M
		2.00 H		0.86 M		0.61 H
15.	1.23 H	0.31 H	0.43 H	0.97 H	0.63 H	0.72 H
		0.43 H		1.50 H		0.09 M
16.	1.64 H	1.46 H	0.46 H	1.78 H	0.39 H	1.07 H
		0.77 H		0.05 M		1.22 H
17.	1.54 H	0.08 M	0.53 H	1.33 H	0.12 H	1.46 H
		2.67 H		0.06 M		0.08 M
18.	7.09 H		0.37 H	0.04 M	0.91 H	1.00 H
				1.84 H		0.08 M
19.			1.27 H	1.46 H	0.37 H	
				0.07 M		
20.		0.63 H		0.61 H	0.46 H	
				0.11 M		
21.				1.27 H	1.56 H	
				0.05 M		

TABLE 2. Mean sizes in mm^2 of hermaphroditic gametophytes. Underline joins means not significantly different, $p < 0.05$ using least significant difference (LSD).

Contaminated SS	Contaminated DS	Untreated DS	Bleach DS	Bleach SS	Untreated SS
2.271	1.397	1.387	0.931	0.715	0.588

contaminated cultures, with DS cultures from all three treatments forming an intermediate size class and SS cultures from bleach and untreated cultures forming the smallest size class (Table 2). In contaminated cultures, H gametophytes from SS cultures were larger than those from DS cultures. In untreated cultures, H gametophytes from SS cultures were smaller than those from DS cultures, and in cultures derived from surface sterilized spores there was no difference in the size of H gametophytes from SS and DS cultures (Table 2).

DISCUSSION

The observation that H gametophytes were larger in DS than in SS cultures for cultures derived from both untreated and sterilized spores, whereas gametophytes were larger in SS than in DS cultures in contaminated cultures, indicates an effect of contamination on the size of H gametophytes. The observation that H gametophytes did not differ in size between DS and SS cultures derived from surface sterilized spores suggests a possible effect of surface sterilization on the size of H gametophytes. However,because there was no difference in size among H gametophytes from DS cultures derived from untreated and surface sterilized spores, and as there was also no difference in size among H gametophytes from SS cultures derived from untreated and surface sterilized spores, we cannot conclude with certainty that surface sterilization had an affect on gametophyte size.

Treatments appear to have affected the antheridiogen response. The greatest antheridiogen response was observed in cultures with no treatment and the least antheridiogen response was observed in contaminated cultures. It is not possible, however, to make any conclusions regarding the effect of surface sterilization, as the 44%cM observed in cultures derived from surface-sterilized spores is intermediate between the 65% cM observed in cultures derived from unsterilized spores and the 29.4% cM observed in contaminated cultures. Surface sterilization of spores may have as much affect on the antheridiogen response as contamination, or may have had no effect. The difference between cultures derived from unsterilized spores and contaminated cultures does indicate, however, that if one wishes to use agar media as a means of limiting the effects of uncontrolled factors in developmental studies, it is important to either prevent contamination or eliminate contaminated cultures from any analysis.

If one concludes that surface sterilization had no effect on the antheridiogen response relative to untreated spores, then surface sterilization was not necessary to eliminate the effects of contamination, and thus is not necessary. If

one concludes that surface sterilization may have affected the antheridiogen response, then surface sterilization may result in the unnecessary addition of a factor affecting gametophyte development, particularly given the lack of any observed contamination in cultures derived from untreated spores. We thus recommend that surface sterilization be avoided unless it is required to prevent contamination.

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Spore Morphology of *Botrychium lunarioides*

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ABSTRACT.—Spore morphology was examined in the rare fern *Botrychium lunarioides* with the scanning electron microscope. Surface topography of the spore walls confirms the placement of *B. lunarioides* in the subgenus *Sceptridium*.

Botrychium is a widespread genus of eusporangiate ferns classified within the family Ophioglossaceae. Clausen (1938) originally divided this genus into three subgenera based on vernation, dissection of the leaf blade, and gametophyte shape. Presently, four subgenera are recognized (Tryon and Tryon, 1982; Tryon and Lugardon, 1991): *Botrychium, Japanobotrychium, Osmundopteris,* and *Sceptridium*. Although some investigators (Kato, 1987 and discussion therein; Sahashi, 1976) have raised these subgenera to generic level, Wagner (1990) considered it best to retain the more conservative classification, at least for the present.

Of particular use in placement of species within the proper subgenus has been spore morphology, in particular surface topography (Tryon and Lugardon, 1991). The scanning electron microscope (SEM) has proven a particularly useful tool for such studies.

Botrychium lunarioides (Michx.) Sw. is a species restricted to the southeastern United States. According to Warren H. Wagner (pers. comm.), "It is one of the rarest ferns in the United States, and not one botanist in a thousand has seen it." However, Watkins (1992) recently completed a status survey of this species in Alabama and while doing so discovered a number of new localities. Also, Do et al. (1996) have shown this species to be more common in eastern Texas than previously thought. The species is considered to belong within the subgenus *Sceptridium* (Wagner and Wagner, 1993). Wagner (1992) gave an analysis of the distinctive features of *B. lunarioides* that differentiated it from other members of subgenus *Sceptridium,* and he placed it in a separate section, *Hiemobotrychium*. Thus, in spite of its distinctive characters, *B. lunarioides* was still considered to belong to subgenus *Sceptridium*. However, no confirmatory evidence from spore morphology has been available. The purpose of this study is to provide that information.

A recent study (Skoda, 1997) recommended placing *B. lunarioides* in a new monotypic genus as *Holubiella lunarioides* (Michx.) Skoda, based on a number of morphological features including tracheidal idioblasts in the leaf stalks. It remains to be seen how this proposal will be received by other pteridologists. For the purposes of the present manuscript, we shall follow the commonly

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accepted view and consider *B. lunarioides* as a species within the genus *Botrychium*.

Materials and Methods

Ripe sporangia were collected in March 1997 from two sites: 1) Pleasant Hill United Methodist Church Cemetery and 2) Claybank Cemetery, both in Ozark, in Dale County, Alabama. The collections were pooled and placed into one-half strength Karnovsky's fixative (Karnovsky, 1965) buffered to a pH of 7.0 in 0.1 M sodium cacodylate for one week. After buffer rinses, the sporangia (and any dislodged spores) were dehydrated in an ethanol series, dried by the critical-point method (Postek et al., 1980), attached to aluminum stubs with double-stick carbon tape, and coated with gold or gold/palladium. Prepared specimens were viewed with a Zeiss Digital Scanning Microscope (DSM) 940 at 10 and 15 kV.

In order to observe the spore wall in sectional view, an additional technique was used. Following initial fixation and buffer washes, spores were postfixed in cold, 1% buffered OsO_4 for 24 hrs. Any unbound osmium was removed by washing in distilled water. The spores were then pelleted and embedded in 2% agar. Small agar cubes containing spores were cut and passed through an ethanol/propylene oxide dehydration series. Infiltration with Spurr's resin (Spurr, 1969) over a period of 48 hrs was followed by final polymerization of the plastic resin overnight at 60°C. Sections of the embedded cells 2–10 μm thick were cut with a glass knife and heat-fixed to circular glass coverslips. The coverslips with attached specimens were passed through a series of chemicals from the Poly/Solv Kit (Polysciences, Warrington, PA), which removed the embedding resin. The coverslips were finally put through two changes of ethanol and allowed to air-dry before being affixed to aluminum stubs, coated, and viewed with the DSM.

Results and Discussion

The spores of *B. lunarioides* generally are trilobate with a trilete scar on the proximal face (Fig. 1). Mean spore diameter along the greatest axis (perispore included) is 32.9 μm (n=25) ranging from 28.5–37.4 μm. The architecture of the spore surface can best be described as rugulate with fusion of the elongate elements. Tryon and Lugardon (1991) summarized the differences in spore topography among the different subgenera thusly: "*Sceptridium*...shallow to prominently rugate, or with more or less fused ridges; *Botrychium*...irregularly papillate to verrucate; *Japanobotrychium*...reticulate with striate ridges; *Osmundopteris*...coarse, distant verrucae." According to their criteria, *B. lunarioides* clearly belongs within the subgenus *Sceptridium*.

The overall shape of the distal face of the spores is slightly convex, whereas the proximal face consists of three flattened, angular surfaces leading away from the trilete scar. These surfaces represent the sites at which the spore was joined to the other three spores of the meiotic tetrad. Detailed investigation of

FIG. 1. Overall view of spores of *Botrychium lunarioides*. FIG. 2. Proximal surface of spore with trilete scar. Note the small openings (arrows) on the proximal surfaces.

these surfaces (Figs. 2, 3) shows the presence of variable numbers of small openings of circular to elliptical shape in the perispore and sporoderm. These openings have a mean diameter of 200 nm (n=30) with a range of 40–650 nm and occur either individually or in small groups. Figure 4 confirms that these openings are confined to the proximal face. Unfortunately, none of the sec-

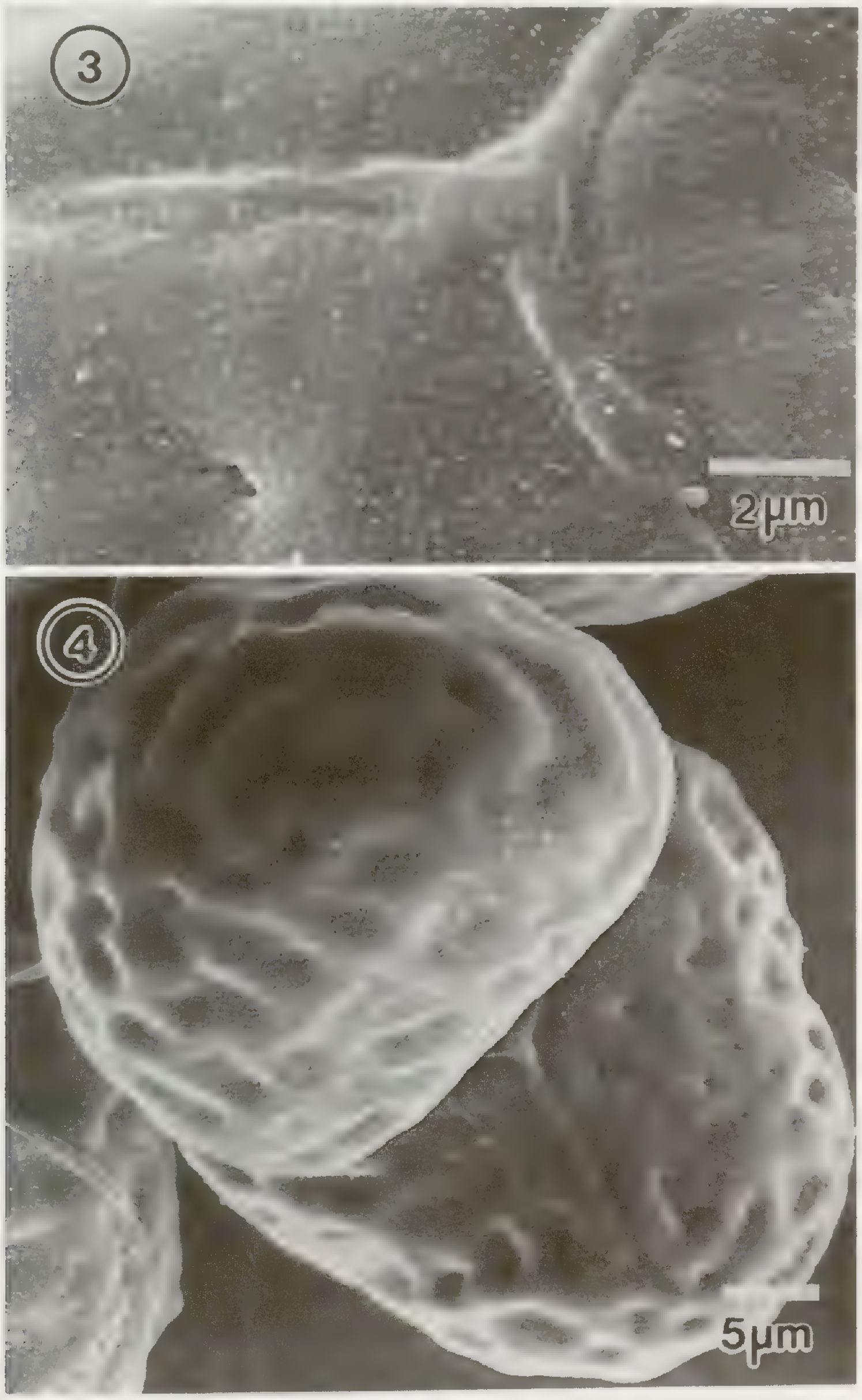

FIG. 3. Detailed view of proximal face of previous spore. Granular nature of the perispore is evident. FIG. 4. View of the proximal face of one spore (lower right individual) and partial distal view of other spore. The small openings are restricted to the area of the spore wall near the trilete scar.

tional views through the sporoderm included any of these structures (Fig. 5), so it is uncertain whether these openings represent the surface of channels passing through the spore wall. It could be hypothesized that these structures represent the remnants of cytoplasmic connections between spores of a given tetrad. Unfortunately, no corroboratory evidence in favor of this view exists. Clearly, they do not represent remnants of cytomyctic channels such as are found between microsporocytes during prophase of meiosis I in anthers (Hes-

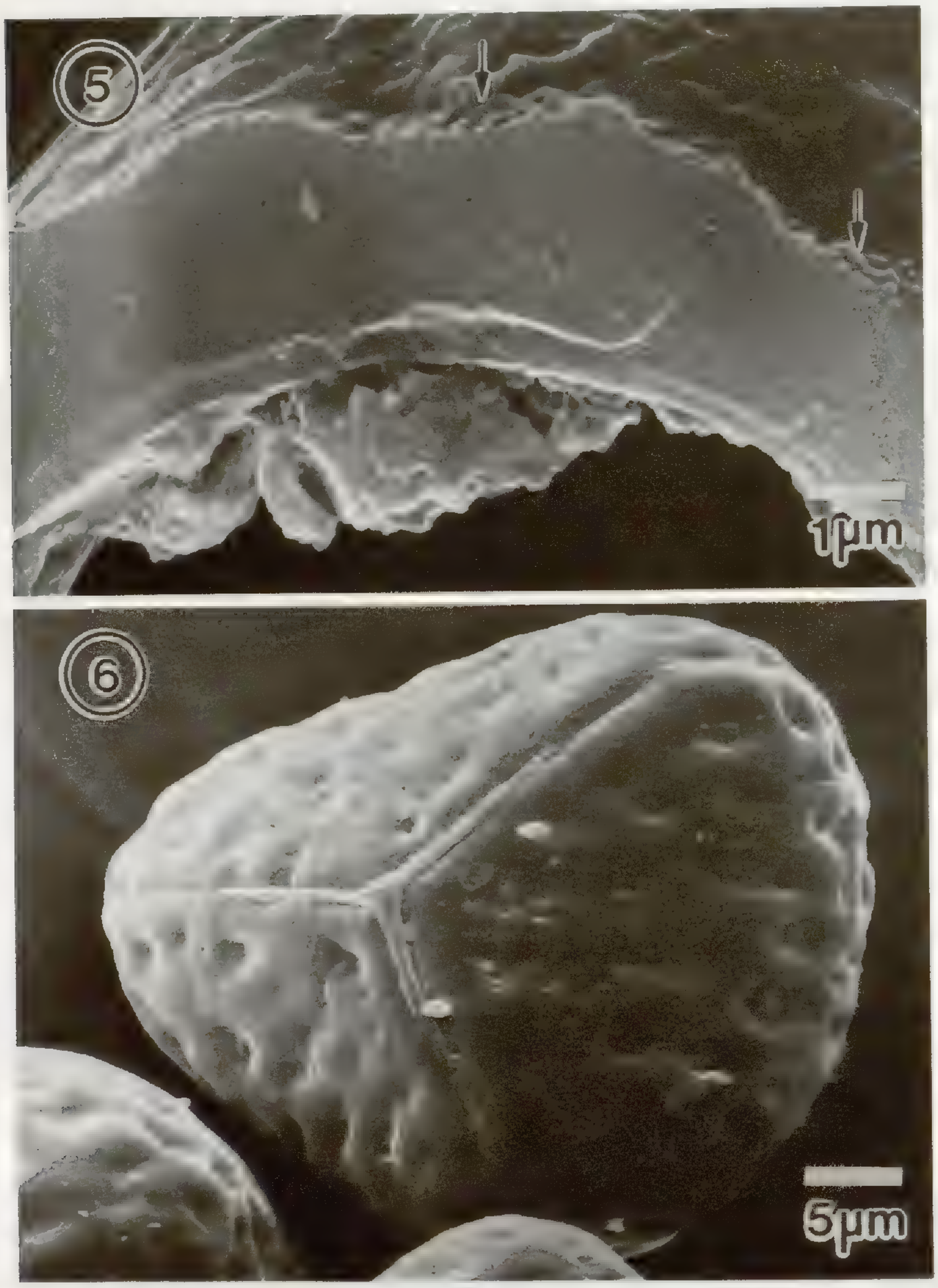

FIG. 5. Sectional view of sporoderm. Arrows denote the granules of the perispore. FIG. 6. Spore without a granular perispore.

lop-Harrison, 1966). Their small diameter and restriction to the proximal walls are proof of this. Although Heslop-Harrison (1966) states that, "No plasmodesmata are ever formed between the daughter cells of either (meiotic) division," there are a few instances (some Winteraceae and orchids) in which the pollen grains of tetrads are connected by cytoplasmic channels through their proximal walls (Heslop-Harrison, 1968; Sampson, 1981). However, a detailed study of spore wall morphogenesis in *B. lunaria* by Pettitt (1979) provided no evidence for cytoplasmic channels.

An alternate explanation for these openings in the proximal surfaces is that they are merely pits and do not penetrate the sporoderm. Sahashi (1976, 1979, 1980) described the proximal surfaces of species of *Botrychium* s. str., *Japanobotrychium* s. str., and *Sceptridium* s. str. as "pitted, punctate, or foveolate." Unfortunately, we were unable to procure his SEM micrographs for compara-

tive purposes. Clearly, more developmental studies of *Botrychium* spores are necessary to clarify this situation.

The nature of the perispore (surface layer) of the spore coat varies from spore to spore. In most instances the surface is quite granular (Figs. 2, 3), whereas in other cases it is very smooth (Fig. 6). Sectional views of the sporoderm show that the granularity, when present, is restricted to the surface (Fig. 5). The remainder of the spore wall (the exoderm) appears homogeneous with the SEM (Fig. 5) although it is known from TEM investigations of other *Botrychium* species that such is not the case (Pettitt, 1979; Tryon and Lugardon, 1991).

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American Fern Journal 88(3):93–105 (1998)

A Millenium and a Half of Fern Illustrations

RICHARD A. RAUH
11 Admiral Lane, Norwalk, CT 06851

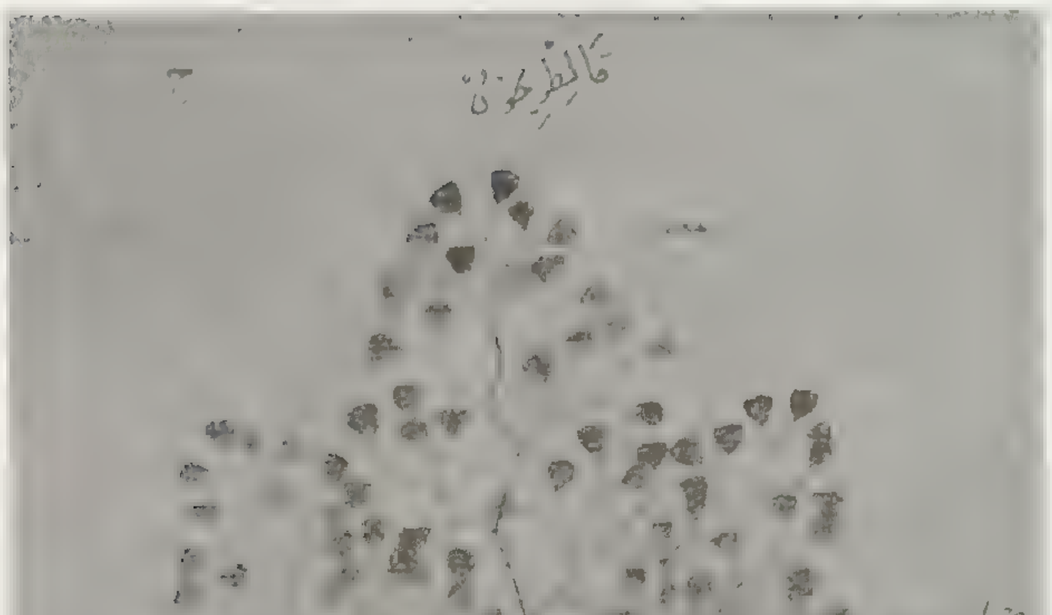
ABSTRACT.—The history of fern illustration is part of the history of western botanical illustration. Drawings of ferns intertwine with the art of the 1500 years this story spans. To concentrate primarily on this history as it is revealed in the published works over this time, an important theme becomes evident. As in so much scientific discovery, technological advances pave the way for a growth of knowledge and more accurate perception. The advances in botanical illustration are balanced between the growing knowledge and talent of the scientific artist and the growing skill of his technician allies, the woodblock cutter, the etcher, the engraver, the lithographer, and the photographer, and the growing strengths of the techniques themselves.

The earliest existence of sound recognizable botanical art is affirmed on the walls of Egyptian tombs. However, no examples of the painted Greek and Roman art that were the source of almost all medieval copies have survived, although it is known from Greek and Roman chronicles that such art did exist.

The earliest botanical volumes that have survived were herbals. They were books about medicinal plants with descriptions and drawings of the plants for identification. Among these plants were pteridophytes thought to have medicinal properties.

At least two species, *Adiantum capillus-veneris* and *Polypodium vulgare*, are in the earliest manuscript dealing with the depiction of plants that has come down to us today. The *Codex Vindobonensis* (Dioscorides, 512 AD), written and illustrated in Constantinople for the daughter of the Byzantine Emperor of the West, is a copy of the Greek text *De Materia Medica* by Dioscorides, a first century AD physician and botanist. It is believed the original work consisted of paintings by Crateuas of some 500 plants, with the written part consisting of little more than their names and healing properties. According to the British art historian Wilfred Blunt, the *Codex* provided "the link between classical and modern botany," and the paintings "the link between ancient Greek and modern European botanical illustration" (Blunt and Stearn 1994). From a facsimile of this text is a lovely, if somewhat primitive, illustration of *Adiantum capillus-veneris* (Fig. 1).

The manuscripts that made up the source of medical and botanical knowledge for the Medieval era were copies of the *Codex* and other classical material. For nearly 1000 years, the manuscripts produced were copies of copies made by illuminators with no or little botanical knowledge so that simplifications, anthropomorphisms, and mistakes made the identification of plants nearly impossible (Apulius, 1481). This problem was compounded because maintaining a fidelity to previously printed words and images was one of the disciplines of Christianity in that period, which found heresy in the observation of nature itself.



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The beginning of the fifteenth century saw the development of the woodcut, a printing process in which the surface of the wood block carries the ink and the background white areas are cut away. This technique was perfected over the next century. At first, the text and illustration were cut from the same block, but as the century progressed the printing press and the invention of moveable type changed this. The earliest printed herbals included illustrations of ferns, at first highly simplified and decorative, but beginning in the *Hortus Sanitatus [Gart der Gesundheit]* (Wonnecke, 1485) to be more recognizable. It was not until two major books of the early sixteenth century were printed that we see the tyranny of the blindly copied image give way at last to a naturalistic, scientific representation. The Brunfels (1530) *Herbarum Vivae Eicones* [Portraits of Living Plants] was the first book that marked this change. It is its artwork drawn by Hans Weiditz (a probable student of Albrecht Dürer) and not so much its text that is remarkable. His drawings (he did not do all the woodcuts) reflect the artist's preoccupation with how things really looked. He even included insect damage, and wilted or damaged leaves. His skillful rendering of *Phyllitis scolopendrium* (Fig. 2), with its meticulously observed and accurately recorded detail, marks a true beginning of modern scientific illustration.

Like Dioscorides, Leonhart Fuchs was a physician-botanist and produced a text, *De Historia Stirpium* (Fuchs, 1542), that was the second of the books whose illustrations marked the major change from medieval copying to original observation. The text is much superior to that of Brunfels in some ways, giving the name, habits, habitats, and uses according to the classical authorities. The plates, closely supervised by Fuchs, were drawn by Albrecht Mayer and show large typical plants, a departure from the individuality of Weiditz', done in delicate but scientifically accurate outline, obviously intended to be colored (a small number of colored copies have survived to this day). Note the early instance of the illustration of dissected details, the rhizome cross-section of *Dryopteris filix-mas* (Fig. 3).

Most of the later works of the 1500s documented medical applications and botanical knowledge but did not mark any major artistic, technical, or scientific advances in the art of illustration. The art of the woodcut, limited not only by the skill of the cutter but also by the restrictions caused by the grain of the wood, reached its peak in the sixteenth century. Metal engraving was introduced a hundred years before it finally began to be applied to botanical illustration. When it did, it surpassed the woodcut, allowing the taxonomic details to be more clearly defined, and the draughtsman's ability to be more accurately reproduced. The two immense volumes of Basil Besler's (1613) *Hortus Eystettensis,* make use of this technique. These volumes (which a contem-

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FIGS. 1–4. Pteridophyte illustrations, 512–1613. 1) *Adiantum capillus-veneris* from the *Codex Vindobonensis*, 512 A.D. 2) *Phyllitis scolopendrium* from Brunfel's *Herbarum Vivae Eicones*, 1530. 3) *Dryopteris filix-mas* from Fuch's *De Historia Stirpum*, 1542. 4) *Ophioglossum [vulgatum]* from Besler's *Hortus Eystettensis*, 1613.

porary critic complained one needed a wheelbarrow to transport) were not an herbal, but a florilegium, a book showing a collection of plant portraits with horticultural rather than scientific or medical emphasis. The Besler book documented the plants in the garden of the Bishop of Eichstatt, and the organization of the folio is based on the seasons in which they bloomed. There are 374 plates, each showing two or three species arranged artistically, with cursive nomenclature. The work included two ferns, *Botrychium lunaria* and *Ophioglossum vulgatum* (Fig. 4).

The seventeenth century also saw the beginning of the translation of the Greek and Latin texts to the vernacular, as in Gerard's (1597) *Herball,* and Parkinson's (1640) *Theatrum Botanicum.* The illustrations in these books, however, relied largely on the reuse of woodblocks from as much as a century before, many probably coming from the storehouse of Plantin Press of Antwerp, and only newly discovered plants required original art (Blunt and Stearn, 1994; Rix, 1981).

Exploration, and documentation of exotic and newly discovered species are reflected in the work of Charles Plumier (1705), a French priest and botanist who made three trips to the West Indies in the years around the beginning of the 18th century. His monograph *Traité des Fougères de l'Amérique* is the first book devoted exclusively to one plant group, in this case ferns of the West Indies. He drew the subjects and engraved the copper plates himself, a rare instance of the artist and technician in one man, and produced scientifically accurate, and decorative illustrations (Fig. 6).

One publication from the mid-eighteenth century (Gesner, 1751) should be mentioned. Jacob Trew, a wealthy physician/botanist of Nuremberg, purchased some of the original drawings and contemporary woodcuts of the great sixteenth century Swiss botanist Conrad Gesner. Gesner published two major works in his lifetime and created 1500 plates for a third work, a history of plants, but died before this could be realized. The art work found its way to various collectors, and some finally reached Trew, who published it two centuries after its conception. Note the elegance and accuracy of Gesner's work in the illustration of *Botrychium lunaria* (Fig. 5).

Toward the end of the eighteenth century, Sir Joseph Banks provided a scientific team to accompany Cook on his first voyage of discovery around the world from 1768 to 1771. The botanical draughtsman in this group was Sidney Parkinson, and during the course of the voyage he completed 955 drawings, 675 sketches, and 230 paintings. Unfortunately for the field of botanical illustration, he died of dysentery on the return trip. Banks had copper engravings made of Parkinson's drawings, but was unable to publish them, and the plates were stored in the basement of the British Museum. A printing of lithographs

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FIGS. 5–8. Pteridophyte illustrations, 1705–1840. 5) *Botrychium lunaria* from Gesner's *Opera botanica,* 1751–1771. 6) *Aspidium triangulum [Polystichum triangulum]* from Plumier's *Traités des Fougéres de l'Amériques,* 1705. 7) *Pteris macilenta* from *Banks' Florilegium,* 1987. 8) *Acrostichum velleum [Cheilanthes vellea]* from Sibthorpe's *Flora Graeca,* 1806–1840.

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copied from the plates was made in the beginning of the twentieth century (Banks et al., 1905), but the plates were again stored in the Museum, and when the German bombing devastated London in World War II they were moved outside in the courtyard and covered with tarpaulins to make room for "more valuable" material. In 1973, thirty plates were published in black and white, including one of the fern *Marsilea* (Banks et al., 1973), and in the 1980s plates were once more cleaned up and used for a limited edition in color known as the *Banks' Florilegium* (1987), which took over seven years to produce. *Pteris macilenta* (Fig. 7) is an example. The exacting hand-colored printing process known as "à la poupée" was employed in this edition. The technique's name comes from the rag-like "dolls" used to apply all the colors to a single plate for a single printing.

There were many more explorations throughout the 18th century, including one made in 1786 by John Sibthorpe (an English botanist) and Ferdinand Bauer (a German-born botanical artist). The voyage was inspired by the *Codex Vindobonensis,* which they studied in Vienna where it still can be seen. They botanized throughout Greece in an attempt to identify the 500 plants mentioned by Dioscorides, and produced what some call the greatest, most beautiful flora of all time (de Bray, 1989), the *Flora Graeca* (Sibthorpe, 1806–1840). Ferdinand Bauer, and later his brother Francis, were the only "stars" of the so-called "golden age" of botanical illustration of the eighteenth century who produced any fern paintings that I could find, but the beauty of Ferdinand's *Acrostichum velleum [Notholaena vellea]* (Fig. 8) from the Sibthorpe flora indicates his mastery of the art, and some plates hint at the heights that botanical illustration had reached. Their botanizing did not turn up the *Adiantum capillus-veneris* by Dioscorides, cited above.

It is hard to overestimate the influence of the work of Linnaeus on botanical knowledge, although some claim his main talent lay in the organization and compilation of other mens' discoveries. He provided a much needed framework for the rapidly growing botanical information of the age, and his work resulted in many botanical artists including details of reproductive organs and fruits in their paintings as well as a more logical organization of the material.

The great German cryptogamist Johannus Hedwig, whose main contribution lay in his work with mosses, demonstrates the growing scientific concern in his approach to ferns. Hand-colored engravings from his *Filices Genera et Species* (1799) show a preoccupation with vegetative and reproductive detail (Fig. 9). John Miller's (1777) artwork for his *Illustration of the Sexual System of Linnaeus* is another case in point. The unidentified *Polypodium [Polystichum*

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FIGS. 9–12. Pteridophyte illustrations, 1777–1844. 9) *Trichomanes [Hymenophyllum polyantha, H. hirsutum]* from Hedwig's *Filicum Genera et Species,* 1799. 10) *Polypodium [Polystichum setiferum?]* from Miller's *Illustratio systematis sexualis Linnaei,* 1777. 11) *Aspidium filix-mas [Dryopteris filix-mas]* from Curtis' *Flora Londinensis,* 1777–1796. 12) *Acrostichum [Elaphoglossum cochleatum, E. didyanium, E.reichenbachii]* from Fée's *Mémoires sur la Famille des Fougères, Mémoire 1,* 1844.

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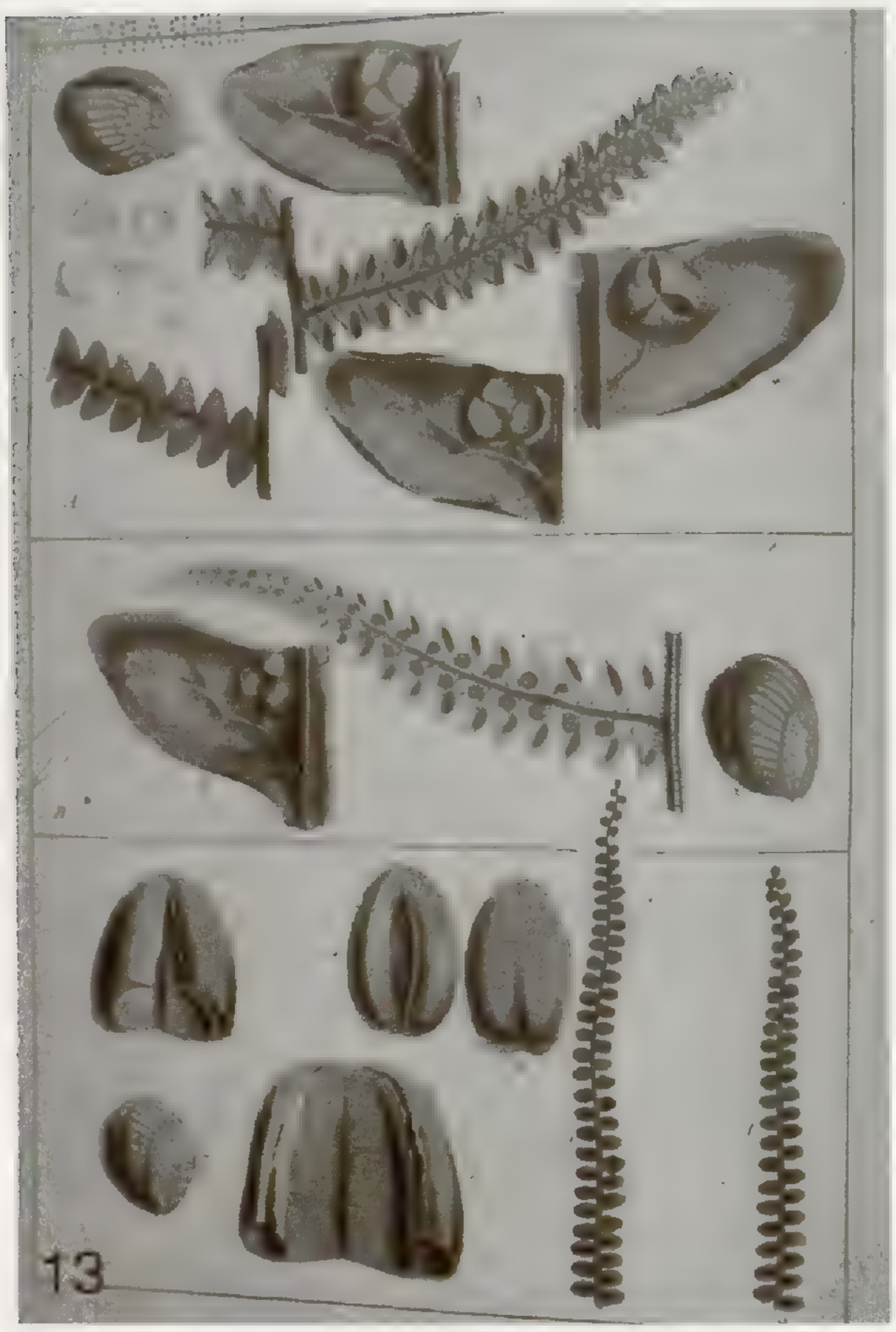
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16 *Adiantum capillus-veneris*

setiferum?] (Fig. 10), illustrates Cryptogamia and portrays one of four orders that were then classified under that heading, Filices, Musci, Fungi, and Algae. The Miller drawings were reproduced by etching, as were those by the self-taught English naturalist James Bolton (1785) in his book *Filices Britannicae.*

Etching is an intaglio process like engraving, in which the ink is forced into the lines, and the plate surface wiped clean to produce the print. Etchings are created by acid eating through those areas exposed by a needle on an acid-resistant coated metal plate. Engravings on the other hand are made by cutting a V-shaped channel in the surface of the metal with a pointed tool called a burin. By far the majority of botanical art of the period was produced by this method, the fineness of the engraving tool more suitable for the precise detail required by scientific illustration. Color was added by hand-painting the printed engraving, with the use of linear crosshatching to create the sense of depth. With the great French flower painter Redouté came the application of stipple engraving to floral work. Employing dots instead of lines on the copper plate gives a much subtler range of tone.

Like the *Flora Graeca* in format, the *Flora Londinensis,* which cataloged the indigenous flowers within a ten mile radius of London, was written by William Curtis (1777–98). Supported by a wealthy patron and getting subscribers to purchase copies, Curtis devoted over ten years of his life to producing these large, expensive, beautifully engraved hand-painted volumes. The artwork was first done by William Kilburn and afterwards by James Sowerby and Syndenham Edwards. An example is the *Aspidium filix-mas [Dryopteris filix-mas]* from the first volume (Fig. 11). The subject matter of the immensely costly work must have been considered weeds or at best local wildflowers and did not attract those who could afford it (Blunt and Stearn, 1994). Sadly, this left Curtis financially strapped. With amazing resilience, he reasoned that a more modest and more affordable product made sense, and in 1787 founded Curtis's Botanical Magazine (Curtis, 1787–1983). The magazine published continuously until 1984, when it was temporarily retitled The Kew Magazine (Royal Botanic Gardens, Kew, 1984–1994). It focussed on pieces about newly discovered or exotic species, with beautiful color illustrations, hand painted until the 1948 edition, and today continues the highest standards of botanical illustration (Royal Botanic Gardens, Kew, 1995 to present). The last fern illustrated was in 1862, at the height of Victorian preoccupation with pteridophytes.

A reproduction technique that had its moment of glory in the mid-nineteenth century was the nature print. In the simplest and earliest version, the actual plant was inked and then put through a press to produce an image, thus destroying the plant. More practical was the development in which the plant

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FIGS. 13–16. Pteridophyte illustrations, 1838–1981. 13) *Gleichenia polypodioides (top), G. microphylla (middle), Platyzoma microphylla (bottom)* from Hooker's *Genera Filicum,* 1838–42. 14) *Woodsia hyperborea [alpina]* from Hooker's *The British Ferns,* 1861. 15) *Asplenium fontanum, A. lanceolatum* from Moore's *The Ferns of Great Britain,* 1855. 16) *Adiantum capillus-veneris* from Abbe's *The Fern Herbal,* 1981.

was pressed between steel and soft lead plates, the lead plate taking the impression. This was electroplated with a thin coating of copper. Type metal was forced into the depressions in the copper plate, and formed the raised image that was used for letterpress relief printing, a process called electrotype. Henry Bradbury's *Asplenium fontanum and A. lanceolatum* (Fig. 15) from Moore's (1855) *The Ferns of Great Britain* is an example of this technique. This process has limitations for complex and solidly three-dimensional plant forms, but in skillful hands was effective, for example, in showing venation patterns in leaves due to their dorsi-ventral form. The technique has since faded from popularity, and is relegated today to the realm of botanical crafts.

The latter half of the nineteenth century brought an increasing scientific and horticultural interest in ferns. Monographs such as the eleven *Mémoires* written by the French botanist Antoine Fée (1844–66) emphasized scientific detail (Fig. 12). In England, William Jackson Hooker, Director of Kew and editor of the Botanical Magazine, was responsible for three major works on pteridophytes that contain splendid illustrations of great scientific and artistic value. His first work on the subject, *Genera Filicum* (Hooker, 1838–42), was illustrated by Francis Bauer who untypically got equal cover credit with Hooker. Francis was the second of the German brothers who made such a major contribution to the art of botanical illustration in the eighteenth and early nineteenth centuries. Although both of these men did their primary work in England, Ferdinand did travel a number of times, notably as a young man accompanying Sibthorpe throughout Greece, whereas Francis remained in Kew for 50 years, turning out a vast quantity of magnificent artwork from materials gathered by others (King, 1978). Toward the end of his life, he illustrated the *Genera Filicum* with a delicacy and attention to detail that is typical of his floral paintings (Fig. 13). Unfortunately, he died before he could complete the work, and another giant of botanical illustration, Walter Hood Fitch, took over.

Fitch was a Scotsman who had been Hooker's protégé from an early age. He had already illustrated much of Hooker's work, as well as the *Botanical Magazine,* was responsible for the lithography of Bauer's drawings, and completed *Genera Filicum* employing Bauer's style. His own, somewhat more flamboyant technique is apparent in the drawing (Fig. 14) from Hooker's (1861) *British Ferns.* Fitch went on to dominate the field of botanical illustration in the nineteenth century, producing the staggering number of over 12,000 published plates in his lifetime (Lewis, 1992).

The nineteenth century thus saw the application of yet another technique of reproduction, lithography, take hold of the printing of botanical books. The technique depends on the repulsion of water and oil; the artwork is drawn on a flat, fine-grained limestone surface with a grease pencil. The drawing itself is removed, leaving an oily residue. The stone is dampened, and then inked. The oil-seeking ink marks only those areas which had been drawn.

The most popular reproduction method during the late Victorian era was chromolithography, an extension of this technique. This color process used as many as thirty registered lithographic stone impressions of different colors to produce a single full-color copy. The technique characterized the vivid and

gaudy popular art and seed catalogues of the period. The illustrations from George Schneider's (1892) *The Book of Choice Ferns* are typical examples.

The early use of photography for fern illustration is in the book *Deutschlands Farne* by Heimrich Waldner (1883). In this first use of the medium for fern illustration, he photographed not ferns in their natural setting but herbarium specimens. Pteridophytes are portrayed in another early photographic book, Blossfeldt's (1929) *Urformen der Kunst,* but here details of *Equisetum hyemale* and fiddleheads of *Adiantum pedatum* are used to show the architectural and ornamental potentialities of plant form. Photography, and especially color photography, has since come to be one of the principal techniques of modern fern book illustration, where the focus is on horticulture or identification.

The twentieth century has produced its own crop of outstanding botanical and scientific illustrators, both in the United States and in Europe. The rich collection of contemporary botanical painting by the Hunt Institute for Botanical Documentation at Carnegie Mellon University in Pittsburgh attests to the continued vitality of the art. Schools of botanical illustration on both sides of the Atlantic are producing highly talented artists. A wealth of beautifully illustrated fern books, floras, guides, and horticultural and scientific works fills contemporary bookshelves. Photographs, pen and ink drawings, and paintings are reproduced by ever more sophisticated techniques that bring to the published works a fidelity to the original art that eluded even the most brilliant earlier technicians. An example of excellent contemporary work is the highly decorative but accurate wood engraving from Elfriede Abbe's (1981) *The Fern Herbal.* The wood engraving employed here is similar to a woodcut, except that the end grain of very hard wood is used, along with a number of specially designed tools. Abbe's *Adiantum capillus-veneris,* (Fig. 16) echoes the illustration we started with from the *Codex Vindobonensis* of nearly 1500 years earlier. The quest for knowledge, the ability to flesh out that knowledge with the pictorial image ever more accurately reproduced, remains a constant.

Acknowledgments

The cited texts are in The LuEsther T. Mertz library of the New York Botanical Garden. I want to express my gratitude to the library staff, and especially to its director, John Reed, for their generosity and cooperation on this project. I also wish to thank Dr. John Mickel of the New York Botanical Garden for his support and scientific guidance. It was at his request that I first prepared a talk for the New York Chapter of the American Fern Society thus initiating this project. I thank the following sources for permission to reproduce art work: *Codex Vindobonensis Mexicanus 1,* Austrian National Library, Vienna, color facsimile edition of the Akademischen Druck-u. Verlagsanstalt, Graz 1974 (Fig. 1); The LuEsther T. Mertz Library of the New York Botanical Garden, Bronx, New York (Figs. 2–6, 8–15); Editions Alecto and British Museum (Natural History) (Fig. 7); from Elfriede Abbe: *The Fern Herbal,* copyright 1985 by Cornell University Press (Fig 16).

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Germination of Spores of the Lycopodiaceae in Axenic Culture

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ABSTRACT.—Spores from four species of *Huperzia*, three species of *Lycopodiella*, and three species of *Lycopodium* were germinated in axenic culture. The ten species included species with subterranean mycorrhizal gametophytes and others with surficial photosynthetic gametophytes. The germination of spores from species with mycorrhizal gametophytes was slow, but much faster than previously reported. It occurred in the dark in a few months for most species instead of years as reported by Bruchmann (1910). Although species of *Lycopodiella* are expected to have rapid germination, spores from two species had slow germination. It appears that not all the species of the Lycopodiaceae with photosynthetic gametophytes exhibit rapid spore germination as reported by Treub (1884).

Germinating the spores of the Lycopodiaceae in axenic culture is difficult. It generally takes a considerable length of time and the percentage of germination is low (Whittier, 1977, 1981; Whittier and Webster, 1986). Efforts over the years to germinate these spores under more natural conditions (i.e., on soil) have demonstrated that spores of the Lycopodiaceae fall into two classes according to how rapidly they germinate (Bierhorst, 1971). The spores of species with subterranean, mycorrhizal gametophytes germinate slowly (Bruchmann, 1910; Barrows, 1935) and those of species with photosynthetic, surficial gametophytes germinate more rapidly (DeBary, 1858 [see Chamberlain, 1917, or Barrows, 1935] and Treub, 1884, 1888).

Studies on the germination of spores from species of the Lycopodiaceae with mycorrhizal gametophytes provide limited information (Table 1). Bruchmann (1910) germinated the spores of *Lycopodium selago* L. (*Huperzia selago* (L.) Bernh. ex Schrank & Mart.), *L. annotinum* L., and *L. clavatum* L. in soil cultures. Barrows (1935) investigated spore germination with *Lycopodium complanatum* L. var. *flabelliforme* Fernald (*L. digitatum* Dill. ex A. Br.) and obtained 16% germination after one year. More recent attempts to germinate these spores have been carried out in axenic culture (Table 1) but less than 0.1% of the spores germinated.

It is probably advantageous for spores forming subterranean gametophytes to germinate slowly and be down in the soil before gametophyte development starts. Indications from earlier studies (Whittier, 1977, 1981; Whittier and Webster, 1986) are that spores producing mycorrhizal gametophytes only germinate in the dark, which would stop germination until the spores are covered by soil. However, why these spores of the Lycopodiaceae need long times in the dark before they germinate and why they have such low percentages of germination is not understood. In other pteridophytes with mycorrhizal gametophytes, spore germination is more rapid and can exceed 50% after a few months in axenic culture (Melan and Whittier, 1990; Whittier and Braggins, 1994).

TABLE 1. Records of growing gametophytes on soil or in axenic culture after spore germination in the Lycopodiaceae since 1850.

Species with	Germination time	Reference
Mycorrhizal gametophytes		
L. selago	3–5 yrs, soil	Bruchmann 1910
L. annotinum	6–7 yrs, soil	Bruchmann 1910
L. clavatum	6–7 yrs, soil	Bruchmann 1910
L. complanatum var. *flabelliforme*	1 yr, soil	Barrows 1935
L. obscurum	1 yr, axenic	Whittier 1977
L. digitatum	1 yr, axenic	Whittier 1981
L. lucidulum	1 yr, axenic	Whittier and Webster 1986
Photosynthetic gametophytes		
L. inundatum	few days, soil	DeBary 1858
L. cernuum	few days, soil	Treub 1884
L. salakense	few days, soil	Treub 1888
L. cernuum	1 mo, axenic	Wetmore and Morel 1951
L. appressum	6 mo, axenic	Peterson and Whittier 1991

Early studies on the germination of spores from species with photosynthetic gametophytes show that these spores germinate rapidly (Table 1). DeBary (1858 [see Chamberlain, 1917 or Barrows, 1935]) and Treub (1884, 1888) germinated the spores of *Lycopodium inundatum* L. (*Lycopodiella inundata* (L.) Holub), *Lycopodium cernuum* L. (*Lycopodiella cernua* (L.) Pic. Serm.), and *Lycopodium salakense* Treub (*Lycopodiella*) on soil. Of these studies using soil, Treub (1888) had the most success by raising sexually mature gametophytes of *Lycopodium salakense*. Spores of *Lycopodium cernuum* were slower to start germinating in axenic culture (Wetmore and Morel, 1951), probably because the nutrient medium contained 2% glucose. Gametophytes of *Lycopodium appressum* (Chapm.) F.E. Lloyd & Underw. (*Lycopodiella appressa* (Chapm.) Cranfill) were grown in axenic culture from spores for a study on the sporophyte/gametophyte junction (Peterson and Whittier, 1991). Although unreported at the time, unexpected results were obtained because very few spores germinated even in the cultures lacking sugar (Table 1).

Because there are problems with spore germination for some species of the Lycopodiaceae in axenic and soil culture, this study was initiated in an effort to understand and to improve germination in this group. Improvements in germination would increase the possibility of growing the gametophytes of the Lycopodiaceae. Because only a small percentage of the gametophytes from this group are known (Bierhorst, 1971; Bruce and Beitel, 1979), making previously undescribed gametophytes available, even from axenic culture, would improve our understanding of the haploid generation of the Lycopodiaceae.

MATERIALS AND METHODS

Spores from the following species were employed in this study: *Lycopodium clavatum* L., *Lycopodium digitatum* A. Braun ex Dill., *Lycopodium obscurum*

L., *Huperzia crassa* (Willd.) Rothm., *Huperzia cumingii* (Nessel) Holub, *Huperzia lucidula* (Michx.) Trevis., *Huperzia saururus* (Lam.) Trevis., *Lycopodiella appressa* (F.E. Lloyd & Underw.) Cranfill, *Lycopodiella caroliniana* (L.) Pic.Serm., and *Lycopodiella cernua* (L.) Pic. Serm. The system of classification followed is that of Øllgaard (1987, 1989). With one exception (*L. cernua*), plants of species from North America were collected in Florida, Massachusetts, and Tennessee. The others were obtained in Ecuador (*H. crassa* and *H. cumingii*), Kenya (*H. saururus*), and Hawaii (*L. cernua*). Vouchers are on deposit in herbaria at VDB, AAU, and AKU.

Spores were obtained by allowing fertile branches or cones to dry over a sheet of paper. The spores from North America were sown within two weeks of their collection and those from other parts of the world were sown within two days of their receipt. The spores were sterilized with 20% Clorox by the method of Whittier (1964), suspended in sterile water, and sown on 15 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were then tightened. The cultures were maintained in darkness or under a 12 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from Gro-lux fluorescent lamps at 20 ± 1°C.

All nutrient media contained, as a final concentration per liter, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, 50 mg K_2HPO_4, FeEDTA, and trace elements. The media were solidified with 1% agar and the pH of the media after autoclaving ranged from 5.0–5.3. Because these spores were being cultured to produce gametophytes for study, there were some differences between the media. The medium employed for spores of species having photosynthetic gametophytes contained 50 mg/L NH_4NO_3 and no sugar. The medium used for spores of species with mycorrhizal gametophytes contained 50 mg/L NH_4Cl and 0.2% glucose. Ammonium chloride was used because the mycorrhizal gametophytes of *Botrychium* (Melan and Whittier 1990) and *Psilotum* (Whittier 1990) grow better in axenic culture if nitrate is not present and there were early indications that this is true for *Lycopodium* and *Huperzia*. The glucose was needed as an energy source for the dark-grown gametophytes.

In some experiments, efforts were made to accelerate the germination of spores from species with mycorrhizal gametophytes. The nutrient medium was not modified, but the spores were pretreated by exposing them to light or cold. For the pretreatments cultures containing spores were maintained under the previously described light conditions or they were placed in a cold room at 5±1°C in the dark.

To compute the germination percentages, spores from several cultures were combined and 500–1000 spores were examined for each determination.

Results and Discussion

The results of this investigation are presented in Tables 2–5. Spores from species with mycorrhizal gametophytes generally germinated slowly in the dark (Table 2). The spores of most species did not germinate until they had been in the dark for three months. Spores of *L. clavatum,* which began to germinate after one month in the dark, were an exception. A second exception

TABLE 2. Germination of *Huperzia* and *Lycopodium* spores in axenic culture. * Indicates a three experiment average—spores from different years were used for each of the three experiments.

Species	Germination						
	Dark						Light
	1 mo	2 mo	3 mo	4 mo	6 mo	9 mo	
*L. digitatum**	—	none	0.9%	—	9.9%	22.5%	none at 9 mo
*L. obscurum**	—	none	0.1%	—	6.3%	34.5%	none at 9 mo
*L. clavatum**	0.07%	7.5%	25.7%	—	44.3%	58.6%	0.3% at 9 mo
H. lucidula	—	—	0.03%	—	0.13%	0.3%	none at 9 mo
H. crassa	—	—	2.6%	23.3%	—	—	4.6% at 4 mo
H. cumingii	—	—	39.0%	95.4%	—	—	8.1% at 4 mo
H. saururus	—	—	none	—	0.4%	1.6%	none at 9 mo

would appear to be those of *H. cumingii,* which exhibited 39% germination at three months and 95% at four months. Because a limited number of *H. cumingii* spores were available, there was only one experiment and no observations earlier than three months were made. Exactly when the first of these spores began to germinate was not determined, but the number germinating at three months is a good indication that it was much earlier than three months. Other than these two species, spore germination was slow, as is expected for species with mycorrhizal gametophytes.

Although germination was slow for most of these species (Table 2), improvements were made over past results. These spores germinated faster than previously reported for both axenic and nonaxenic conditions. They germinated in a few months rather than years as Bruchmann (1910) reported. The final germination percentages were higher than previously obtained in axenic culture (Whittier, 1977, 1981) and also for the nonaxenic conditions used by Barrows (1935).

Contrary to the conclusions in the previous paragraph, germination of *H. lucidula* spores showed no significant improvement (Table 2) compared with the previous study (Whittier and Webster, 1986). Even though the results reported in Table 2 are for one experiment, many attempts to increase the germination of these spores were carried out over several years. The germination percentages in the other experiments were no better than those in Table 2. It appears that conditions that produce moderate germination for several species do not promote the germination of *H. lucidula* spores. Whether these spores are inherently slow to germinate or conditions not employed in this study are necessary to stimulate germination has not been resolved.

With one exception, a species of *Tmesipteris,* spores that give rise to mycorrhizal gametophytes have not germinated in illuminated axenic culture (Whittier and Braggins, 1994). In this study there does not appear to be such an all or none effect (Table 2). Spores of three of the seven species germinated in the light. However, fewer of these spores germinated in light than in dark and rarely did macroscopic gametophytes develop in illuminated cultures. Even though it cannot now be reported that spores of the Lycopodiaceae that

TABLE 3. Effect of pretreatments on the germination of *Lycopodium* spores.

Species	Pretreatment	Percentage germination		
		At end of 3 mo pretreatment	At end of 3 mo of dark after pretreatment	At end of 6 mo of dark after pretreatment
L. obscurum	cold	none	13.3%	33.5%
	light	none	49.3%	64.8%
	dark	3.3%	36.3%	68.6%
L. digitatum	cold	none	6.8%	21.8%
	light	none	0.3%	4.4%
	dark	0.2%	3.6%	21.1%
L. clavatum	cold	none	81.5%	—
	light	none	93.5%	—
	dark	6.1%	50.3%	—

give rise to mycorrhizal gametophytes never germinate in the light, it certainly appears that germination in the light is atypical for these spores.

Bruchmann (1910), who waited for up to seven years for *Lycopodium* spores to germinate, commented on the possibility that there might be conditions which would accelerate the germination of spores from species with mycorrhizal gametophytes. In an attempt to examine this possibility, spores of four species that could be easily obtained were given three month pretreatments of light or cold in an effort to stimulate their germination when placed under the normal dark conditions (Table 3). No germination occurred during either of the three month pretreatments. The spores of *H. lucidula* were subjected to the pretreatments, but they are not included in Table 3 because the pretreatments had no effect on their germination.

The cold pretreatment inhibited the germination of *L. obscurum* spores when placed under the normal dark conditions (Table 3). The light pretreatment did stimulate germination to some extent after three months in the dark, but had no effect after six months of darkness. The effect was different for *L. digitatum* spores (Table 3). The light pretreatment delayed germination and the cold pretreatment accelerated germination after three months in the dark, but had little effect after six months in the dark. These results suggest that pregermination changes were occurring in the spores in the cold for *L. digitatum* and in the light for *L. obscurum*. However, for both *L. obscurum* and *L. digitatum* spores there was no real advantage in using these pretreatments because germination at nine months was no better than obtained with normal cultural conditions in the dark.

The spores of *L. clavatum* responded to both pretreatments (Table 3). The percent germination after either of the three month pretreatments followed by three months in the dark was more than tenfold higher than for spores placed in the dark for three months. The germination was also much higher after either sequence of pretreatment followed by three months in the dark than that obtained with spores maintained in the dark for six months. It would appear that pregermination changes occurred faster with the cold or light pre-

TABLE 4. Effect of 1 or 2 month light pretreatment on the germination of spores of *Lycopodium clavatum*.

Light pretreatment	Germination at end of dark period after light pretreatment			
	1 month	2 months	3 months	4 months
None (dark)	0.05%	8.2%	30.4%	41.5%
1 month	12.5%	62.2%	—	—
2 months	36.6%	73.1%	—	—

treatments than under the normal dark conditions. This allowed for the rapid germination of the pretreated spores of *L. clavatum* once they were placed in the dark under the normal conditions.

Because the three month pretreatments promoted the germination of *L. clavatum* spores, additional tests were carried out to determine if shorter light pretreatments had any effect. The spores were pretreated with light for one or two months and then transferred to the dark (Table 4). Both pretreatments were sufficient to accelerate germination once the spores were placed in the dark. Whether the germination percentages are compared on a total time basis (time in light plus that in dark) or only on a dark basis, the pretreated spores germinated to a greater extent than spores placed immediately in dark culture. For example, the spores pretreated with light for one month and followed by one month of darkness germinated to a greater extent than spores placed in the dark for one or two months. The germination of spores placed in light for one month and followed by two of darkness was almost four times higher than that for spores placed in the dark for two months and twice as high as those placed in the dark for three months. The pregermination changes that precede spore germination occurred during the light pretreatments, because after one month in the dark more spores pretreated with light for one or two months germinated (12.5% and 36.6% respectively) than the 0.05% germination that occurred with the spores placed in the dark for one month.

The pretreatments are especially effective in promoting the germination of *L. clavatum* spores. With one month of light followed by two months of darkness, 62% of the spores germinated at the end of 3 months compared to the 6–7 years reported by Bruchmann (1910). With the other species there was a smaller effect and variation as to which of the pretreatments were effective. The spores of *H. lucidula* did not respond to either pretreatment. Whether modifications to the pretreatments (light intensity, temperature, or duration) are necessary to make them more effective in accelerating spore germination in other species has not been examined. However, the effectiveness of pretreatments with spores of some species suggests that they might also promote spore germination in other species with mycorrhizal gametophytes.

Not all the spores of the Lycopodiaceae that form photosynthetic gametophytes gave the expected results (Table 5). The spores of *Lycopodiella cernua* germinated as rapidly as has been reported by Treub (1884) and over 97% of them germinated in 3 weeks. Spores of the other two species sown did not

TABLE 5. Germination of *Lycopodiella* spores in axenic culture.

	Germination								
	Light								Dark
	1 wk	2 wk	3 wk	1 mo	2 mo	3 mo	6 mo	1 yr	1 yr
L. cernua	0.1%	44.2%	97.3%	—	—	—	—	—	none
L. carolinianum	—	—	—	—	0.04%	—	0.6%	1.0%	none
L. appressa	—	—	—	0.4%	—	2.5%	5.7%	8.2%	none

germinate as rapidly (Table 5). The first germination was at two months for *L. caroliniana* spores and one month for those of *L. appressa.* The remaining spores of both species germinated slowly. Only 1% of the *L. caroliniana* spores and 8.2% of the L. *appressa* spores had germinated after one year. Besides being slower to germinate than *L. cernua* spores, they were slower to germinate than the spores of several of the species with mycorrhizal gametophytes (Table 2). Only the spores of *H. lucidula* and *H. saururus* were slower to germinate than spores of these two species. To eliminate any possibility that dark conditions might stimulate germination, cultures with *L. cernua, L. caroliniana,* and *L. appressa* spores were placed in the dark, but no germination occurred (Table 5). Gametophytes raised from the slow germinating spores of *L. appressa* and *L. caroliniana* had the normal morphology and the ability to undergo sexual reproduction. Sporophytes have formed on *L. appressa* gametophytes (Peterson and Whittier, 1991) and those of *L. caroliniana* in older cultures.

Difficulties in germinating the spores of the Lycopodiaceae have not been completely resolved by this study. Very few spores from some species germinate after extended periods of time. Any correlations of spore germination with the type of gametophyte produced by a species does not appear as clear-cut as previously thought. Under the conditions of axenic culture, spores from some species with photosynthetic gametophytes can be slower to germinate than spores from some species with mycorrhizal gametophytes. With most of the species having mycorrhizal gametophytes, spore germination is slow, especially if compared with that of most other pteridophytes, however with these techniques it is much faster than previously reported. The success of pretreatments with *L. clavatum* spores does allow for hope that conditions as yet unknown will be found to promote and accelerate spore germination in other species. Besides accelerating germination in many species, the use of axenic culture has provided the opportunity to grow the previously undescribed gametophytes of *H. crassa, H. cumingii,* and *H. saururus.*

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Fern Spore Morphology and Spore Rain of a Preserved Cerrado Region in Southeast Brazil (Reserva Biológica e Estação Experimental de Moji Guaçu, São Paulo)

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ABSTRACT.—This paper presents a morphological and phenological study of the spores of the fern species of a preserved cerrado area, the Reserva Biológica e Estação Experimental de Moji Guaçu (São Paulo State), Brazil. Of the 40 fern species found in the Reserve, 9 occur in the cerrado, 35 in the gallery forest that surrounds the cerrado, and four species are common to both. Twenty-two species have monolete spores and 18 trilete spores. Spore rain was collected monthly at four localities: open cerrado, cerrado, gallery forest, and marsh. Spores of 16 species were present in the spore rain. The highest percentage of spores was found in the marsh and the lowest in the open cerrado. In all four localities, the highest percentage of spores was observed in the summer months of January and February.

Pteridophyta can be found in all types of habitats (Raghavan, 1991), including very dry regions that may be subjected to periodic fires. The cerrado, Brazilian savanna, originally covered about 22% of the country, extending from the Equator to the Tropic of Capricorn. Much of the cerrado is subjected to prolonged and often severe winter drought lasting up to four months (Alvim and Araujo, 1952; Eiten, 1972).

Esteves and Felippe (1985) described nine species of pteridophyta in the cerrado of the Reserva Biológica de Moji Guaçu, São Paulo state: *Adiantum serratodentatum* Willd., *Anemia flexuosa* (Sav.) Sw., *A. raddiana* Link, *Doryopteris concolor* Langsd. & Fisch., *Microgramma squamulosa* (Kaulf.) de la Sota, *Pleopeltis angusta* Humb. & Bonpl. ex Willd., *Polypodium hirsutissimum* Raddi, *P. latipes* Langsd. & Fisch., *P. polypodioides* (L.) Watt var. *minus* (Fée) Weath. These nine fern species are either drought resistant or the sporophytes are present only during the wet season. This cerrado area surrounds a gallery forest in which 35 fern species were found by Simabukuro (1995). Simabukuro et al. (1994) mentioned 36 species, but it was discovered later that *Thelypteris linkiana* (C. Presl) R.M. Tryon had been mistakenly identified; also *Thelypteris brevisora* (Ros.) Ponce is now known as *Thelypteris dutrai* (C.Chr. ex Dutra) Ponce.

Four cerrado species were also present in the gallery forest: *Microgramma squamulosa, Pleopeltis angusta, Polypodium latipes,* and *P. polypodioides* var. *minus*. Thus, 40 species have been identified in the Reserva. Simabukuro et

al. (1994) mentioned that *Dicranopteris flexuosa* (Schrad.) Underw., *Pityrogramma trifoliata* (L.) R.M. Tryon, and *Pteridium aquilinum* (L.) Kuhn var. *arachnoideum* (Kaulf.) Brade were invaders (weeds) of the gallery forest. In the marsh around the gallery forest were found *Lycopodiella cernua* (L.) Pichi-Serm., *Pityrogramma calomelanos* (L.) Link, *Thelypteris interrupta* (Willd.) Iwats., and *Thelypteris serrata* (Cav.) Alston.

Only a few papers have dealt with spores of pteridophyta in pollen rain in Brazil: They were detected in very small quantities during August–October in a cerrado area in Aparecida, Goiás (Salgado-Labouriau, 1973); they were also detected near the center of the city of Rio de Janeiro, with a predominance of *Adiantum* (Barth, 1975).

This paper presents: 1) a detailed morphological study of the spores of the fern species from the gallery forest; 2) use of this spore data plus the morphological study previously published (Esteves and Melhem, 1992) to correlate the fern species of the Reserva Biológica de Moji Guaçu with spore rain analyses over a period of one year. This is the first study of this kind carried out in Brazil.

Methods and Materials

Study Area (Fig. 1).—All 35 fern species were collected 1984–1994 on monthly visits to the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu (Simabukuro et al., 1994; Simabukuro, 1995). The species were identified by Dr. Paulo Windish (São José do Rio Preto, SP), Alexandre Salino (Belo Horizonte, MG), and Dr. Jefferson Prado (São Paulo, SP). The Reserve (22°18′S, 47°11′W) is located in Moji Guaçu, State of São Paulo, Brazil (Eiten, 1963). The gallery forest is situated along the Cortado and Fundão streams. In addition to the gallery forest, the Reserve includes cerrado and marsh, and is surrounded by *Pinus* sp. plantations (Mantovani and Martins, 1993). In the cerrado, nine fern species occur (Esteves and Felippe, 1985). Forty total species of pteridophyta occur in the Reserve.

Spore Morphology.—The morphology of the spores of the nine fern species that occur in the cerrado were studied previously (Esteves and Melhem, 1992). Of the gallery forest species, the majority of the spores are from herbarium specimens collected in the Reserva Biológica de Moji Guaçu by Aldo Klein (AK), Eliana Akie Simabukuro (EAS), and Luciano Mauricio Esteves (LME), which are deposited in the herbarium of Universidade Estadual de Campinas, Brazil (UEC). The spore morphology of 31 species was studied (Table 1). Of the 35 species mentioned in Simabukuro (1995), five species (*Microgramma squamulosa Pleopeltis angusta, Polypodium hirsutissimum, Polypodium latipes, P. polypodioides* var. *minus*) that also occur in the cerrado have been studied previously (Esteves and Melhem, 1992).

The spores were treated by the acetolysis method (Erdtman, 1971) with the period of treatment adjusted for each species. The slides were gelatin-glycerin mounted according to Kisser (Erdtman, 1971). The spores were measured in

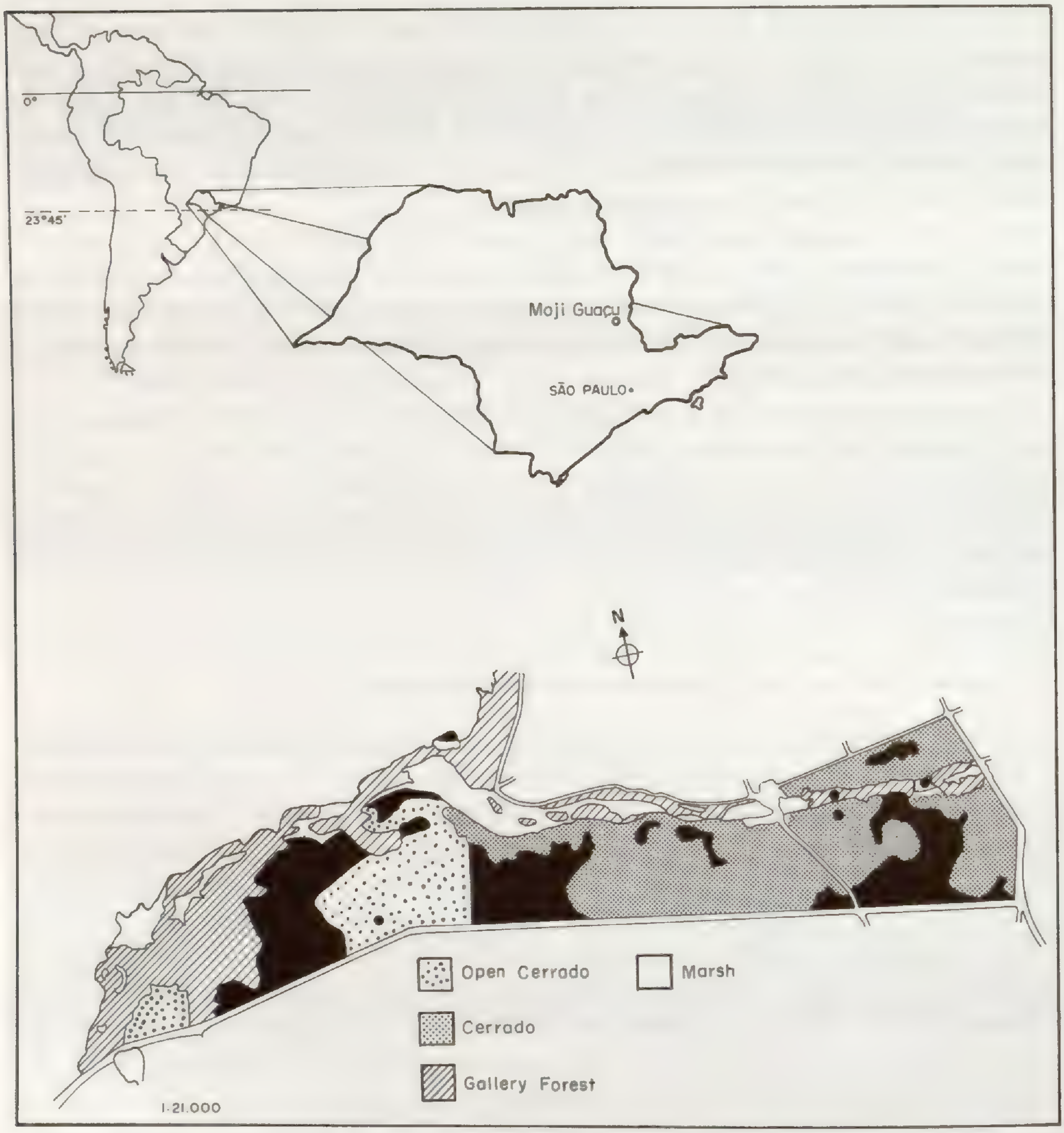


FIG. 1. Estação Experimental e Reserva Biológica de Moji Guaçu (São Paulo State), Brazil. Closed circles indicate locations of spore-rain collectors.

polar and equatorial views (25 spores) and the thickness of the wall also was measured (10 spores) as recommended by Salgado-Labouriau (1973). The sample mean ($\bar{X}$), standard error of mean ($S_{\bar{X}}$), sample standard deviation (S), coefficient of variation (VC), and range (the smallest and largest spores) were calculated for each species in each view (Snedecor, 1962). A photomicroscope (Olympus Vanox) was used to photograph the spores. In some instances, to study the perispore, a scanning electron microscope (Cambridge S90B) also was used. In this case, the spores were not submitted to acetolysis. They were sputter-coated with gold for 3 min (10nm thickness). In this paper, photographs are shown only when they are not presented in Tryon and Lugardon (1991).

TABLE 1. Specimen data for ferns species used in spore morphology. Abbreviations for major collectors: Aldo Klein (AK), Eliana Akie Simabukuro (EAS), Luciano Mauricio Esteves (LME).

Aspleniaceae	
Asplenium serra Langsd. & Fisch.	12-vii-89, EAS 13; 11-x-89, EAS 35.
Blechnaceae	
Blechnum brasiliense Desv.	07-vi-89, EAS 4; 12-vii-89, EAS 9.
B. glandulosum Link	12-vii-89, EAS 12.
B. raddianum Ros.	23-v-91, EAS 86.
B. serrulatum L.C. Rich.	1922, SP 21741 (SP).
Salpichlaena volubilis (Kaulf.) Hook.	31-v-85, AK 47; 27-ix-89, EAS 15.
Cyatheaceae	
Cyathea delgadii Sternb.	07-vi-89, EAS 7.
C. phalerata Mart.	08-xi-89, EAS 53.
Dennstaedtiaceae	
Lindsaea lancea (L.) Bedd.	31-v-85, AK 46; 12-vii-89, EAS 14.
L. quadrangularis Raddi	07-vi-89, EAS 8; 12-vii-89, EAS 11.
Pteridium aquilinum (L.) Kuhn var. *arachnoideum* (Kaulf.) Brade	09-v-85; LME 68.
Dryopteridaceae	
Cyclodium meniscioides (Willd.) C. Presl var. *meniscioides*	07-vi-89, EAS 5; 27-ix-89, EAS 19.
Gleicheniaceae	
Dicranopteris flexuosa (Schrad.) Underw.	09-v-85, LME 67; 23-v-91, EAS 87.
Hymenophyllaceae	
Trichomanes cristatum Kaulf.	21-vii-91, *A. salino 958* (UEC).
T. emarginatum C. Presl	09-v-85, LME 72; ii-29, *A.C. Brade* s.n. (SP).
Lycopodiaceae	
Lycopodiella cernua (L.) Pic.-Serm.	08-v-85, LME 65.
Osmundaceae	
Osmunda cinnamomea L.	30-v-85, AK 42.
O. regalis L.	31-x-91, EAS 88.
Polypodiaceae	
Campyloneurum phyllitidis (L.) C. Presl	07-vi-89, EAS 2.
Polypodium fraxinifolium Jacq.	09-v-85, LME 71; 07-vi-89, EAS 1.
P. ptilodum Kunze	27-ix-89, EAS 18.
Pteridaceae	
Adiantum fructuosum Spreng.	31-x-91, EAS 93.
Pityrogramma calomelanos (L.) Link	08-v-85, LME 64.
P. trifoliata (L.) R.M. Tryon	24-iv-46, *W. Hoehne* s.n. (SP).
Thelypteridaceae	
Thelypteris chrysodioides (Fée) Morton	07-vi-89, EAS 3.
T. dentata (Forsk.) E.P. St. John	16-iv-93, EAS 97.
T. dutrai (C. Chr. ex Dutra) Ponce	16-iv-93, EAS 95.
T. interrupta (Willd.) Iwats.	08-v-85, LME 63; 25-xi-92, *E.L. Catharino, I. Cordeiro, W. Ribeiro, C. Simonetti & C. Machado 1723* (SP).
T. longifolia (Desv.) R.M. Tryon	09-v-85, LME 69.
T. rivularioides (Fée) Abbiatti	25-iv-90, EAS 83.
T. serrata (Cav.) Alston	18-iv-85, AK 27.

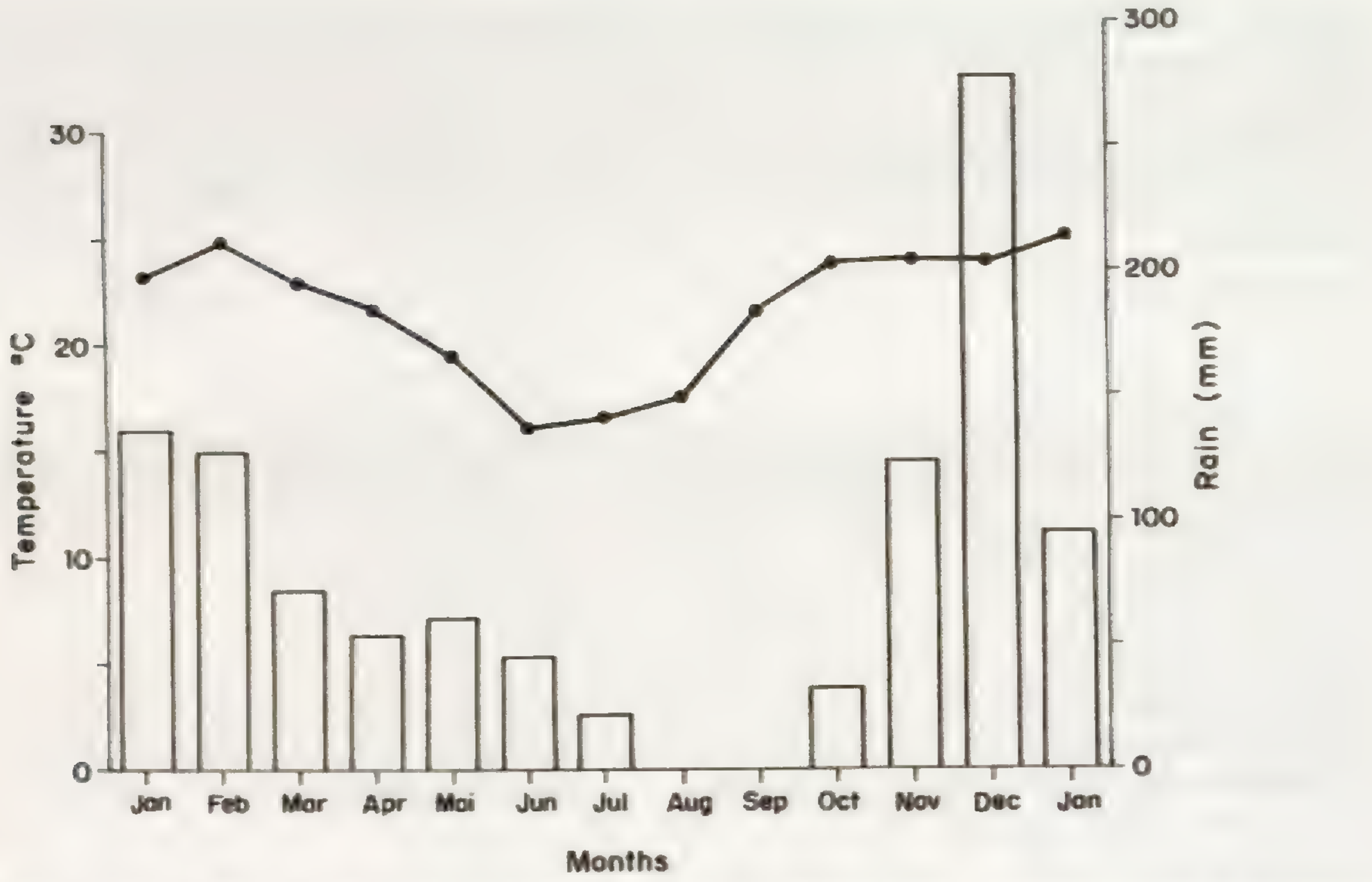


FIG. 2. Temperature (°C) and rain (mm) between January 1994 and February 1995 at Reserva Biológica e Estação Experimental de Moji Guaçu (São Paulo state), Brazil. Open bars = rain; closed circles = temperature.

SPORE RAIN ANALYSIS.—Aerial plastic collectors (9cm diameter and 2 liter capacity) containing 100 ml glycerin were placed at 1.5m from the soil surface as described by Melhem and Makino (1978). They were placed in four different points in the Reserve: gallery forest, marsh, cerrado, and open cerrado (Fig. 1). The collectors were checked monthly for a year. In the present study, the analysis of spore rain was done from January 1994 to February 1995; no analysis was done for February 1994. Rainfall and temperature at the Reserve during the period of the experiment is presented in Fig. 2. Material from each collector was concentrated and treated by acetolysis. At least 3 slides and a total of 500 grains (spores + pollen) were counted per collector for each round of sampling. The spores were identified and their percentage in each sample was calculated.

RESULTS AND DISCUSSION

SPORE MORPHOLOGY.—Short descriptions of monolete spores are included in Table 2. The measurements of the monolete spores are presented in Table 3 (polar and equatorial axes), Table 4 (length and margin of the scar), and Table 5 (exospore measurements).

It should be noted that spores of *Asplenium serra* are dark-brown (when fresh); thus, it was not possible to measure the exospore because of the dark and thick perispore. Also, the perispore of *A. serra* has been reported as fenestrate, according to Puttock and Quinn (1980), but reticulate, according to

Tryon and Tryon (1982) and as seen in samples from the Dominican Republic (Tryon and Lugardon, 1991).

The measurements of the spores of *Blechnum brasiliense* presented here agree with the data both from Erdtman and Sorsa (1971) for spores collected in Brazil and Morbelli (1976) for spores from Canela, RS, Brazil. The granulate perispore was also observed by Nayar *et al.* (1966) and Tryon and Lugardon (1991). The three-layered perispore observed by Erdtman and Sorsa (1971) was not observed here. According to Tryon and Lugardon (1991), the perispore of *Salpichlaena volubilis* is easily removed; in the present material the perine resisted acetolysis. The presence of globules in the species of *Blechnum* studied here is very common in Polypodiaceae, but rare in Blechnaceae.

It was not possible to measure the exospore of *Cyclodium meniscioides* var. *meniscioides* because of the very dark and thick perispore. The measurements and description agree with Smith (1986). In relation to the exospore, the results are very similar to the ones obtained by Kremp and Kawasaki (1972) for spores from Peru. These results agree in the exospore being psilate and differ from the observations of Erdtman and Sorsa (1971), in which the exospore was granulate.

The verrucate exospore of *Campyloneurum phyllitidis* is different from the exospore with tubercles seen in *Microgramma squamulosa* but very similar to *Pleopeltis angusta* (Esteves and Melhem, 1992). The verrucae are very low, whereas according to Nayar and Devi (1964) the exospore presents areolae or is subpsilate according to Kremp and Kawasaki (1972). The spores in the present study are smaller than the ones presented by Kremp and Kawasaki (1972) and Nayar and Devi (1964), but similar to Pal and Pal (1970). The perispore of spores of *Polypodium fraxinifolium* is easily removed by acetolysis; ornamentation decreases towards the scar. The measurements presented here are similar to the ones presented by Nayar and Devi (1964). The perispore of *P. ptilodon* is not observed in fresh and acetolysed spores; verrucae decrease in size towards the scar. The measurements presented here are slightly larger than the ones presented by Oliveira-Camargo and Ferreira (1976).

Thelypteris chrisodioides presents a dark-brown perispore, which can be removed with 2 min of acetolysis. It was not possible to measure the exospore of *T. dentata*; according to Tryon and Tryon (1982) the perispore of this species presents large conspicuous folds, which agrees with the present results. Spores of *T. dutrai* and *T. interrupta* were seen with and without perispore following 2min of acetolysis. It was not possible to measure the exospore of *T. longifolia*. In spores of *T. rivularioides*, it was not possible to measure the scar because of the very dense, dark and strongly adherent perispore. In *T. serrata*, it was not possible to measure the exospore because the perispore could not be removed even by 5 min of acetolysis.

TRILETE SPORES.—Short descriptions of trilete spores are included in Table 6. The measurements of the trilete spores are presented on Table 7 (polar and equatorial axes), Table 8 (length and margin of the scar), and Table 9 (exospore measurements).

TABLE 2. Morphology of monolete fern spores in the study. Abbreviations: E = equatorial view; OM = optical microscopy; Ex = Exine; Pe = perine; SM = scanning electron microscopy.

Species	Size	Shape	Ornamentation
Aspleniaceae			
Asplenium serra	medium	ellipsoidal E: plane-convex	Pe: reticulate to fenestrate; dark and thick; strongly adhered (not removed by 2.5 minutes acetolysis) Ex: psilate; presents two layers: the interior one being darker and thinner than the external one
Blechnaceae			
Blechnum brasiliense	medium	ellipsoidal E: plane-convex	Pe: flimsy; granulate with sparse deposition of globules and rugule (small, low ridges); strongly adhered in immature spores (not removed by 2–6 min acetolysis and 45 NaOH) Ex: psilate; two layers
B. glandulosum (Fig. 3A)	medium	ellipsoidal E: concave-convex	Pe: flimsy; scabrate with sparse globules Ex: psilate in OM and granulate in SM
B. raddianum (Fig. 3B)	large	ellipsoidal E: plane-convex	Pe: thin, partially fragmented in acetolysed spores; presents sparse projecting folds; granulate (OM) and with adhered rodlets under SM (most of it removed by acetolysis) Scar: developed margin
B. serrulatum	medium	ovate E: plane-convex	Pe: thin; with or without globules Ex: psilate; two layers.
Salpichlaena volubilis	large	ellipsoidal E: plane-convex	Pe: thin and granulate with irregular deposits of large number of spherules Ex: psilate; two layers. Scar: developed margin
Dryopteridaceae			
Cyclodium meniscioides	medium	ellipsoidal E: plane-convex	Pe: strongly adhered, granulate; echinate, winglike folds Ex: psilate

TABLE 2. Continued.

Species	Size	Shape	Ornamentation
Polypodiaceae			
Campyloneurum phyllitidis	medium	ellipsoidal E: plane-convex	Pe: thin; translucid, with small and sparse globules Ex: verrucate, but with low verrucae; with circular shape
Polypodium fraxinifolium (Fig. 3C)	large	E: plane-convex to oblate	Pe: thin; sparse grains Ex: verrucate, verrucae of irregular shapes to tubercles; two layers
P. ptilodum (Fig. 3D)	large	ellipsoidal E: plane-convex	Pe: absent Ex: verrucate, with elevated verrucae; two layers Scar: developed margin
Thelypteridaceae			
Thelypteris chrysodioides (Fig. 3E, 3F)	medium	ellipsoidal E: plane-convex	Pe: with short folds; spiculae and pilae in great number over the folds Ex: psilate (OM) and granulate (SM); two layers Scar: developed margin
T. dentata (Figs. 3G, 3H)	large	ellipsoidal E: plane-convex	Pe: large folds; irregular distribution of spiculae Ex: psilate Scar: developed margin
T. dutrai (Fig. 3I)	large	ellipsoidal E: plane-convex	Pe: reticulate with gemmae in the union of the walls Ex: psilate; two layers Scar: developed margin
T. interrupta (Fig. 3J)	large	ellipsoidal E: plane-convex	Pe: large folds; irregular distribution of spiculae Ex: psilate; two layers Scar: with thin margin
T. longifolia (Fig. 3K)	large	ellipsoidal E: plane-convex	Pe: large folds with spiculae Ex: psilate Scar: developed margin
T. rivularioides (Fig. 3L)	large	ellipsoidal E: plane-convex	Pe: reticulate Ex: psilate
T. serrata (Fig. 3M)	medium	ellipsoidal E: plane-convex	Pe: large folds with spiculae Ex: psilate

TABLE 3. Measurements of polar and equatorial axes of monolete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu.

Species	Polar axis				Larger equatorial axis				Lesser equatorial axis			
	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)
Aspleniaceae												
Asplenium serra	18.5–35.3	27.7 ± 1.6	3.9	14.6	35.8–51.7	43.8 ± 1.4	3.5	8.1	24.6–37.4	29.9 ± 1.0	2.0	8.3
Blechnaceae												
Blechnum brasiliense	20.9–33.7	28.0 ± 1.2	3.0	10.7	39.3–46.7	43.2 ± 0.8	1.9	4.5	24.6–31.8	28.1 ± 0.8	2.1	7.5
B. glandulosum	23.2–32.4	27.7 ± 1.1	2.6	9.4	36.0–49.5	40.4 ± 1.4	3.5	8.6	23.1–28.1	25.8 ± 0.5	1.2	4.8
B. raddianum	39.7–51.2	44.6 ± 2.2	3.4	7.6	55.5–67.4	61.1 ± 1.6	3.9	6.3	41.2–49.7	43.7 ± 1.8	2.5	5.7
B. serrulatum	20.5–32.3	26.5 ± 2.8	3.9	14.8	35.5–67.4	38.5 ± 4.6	2.2	5.7	—	—	—	—
Salpichlaena volubilis	52.0–81.9	64.4 ± 3.8	9.3	14.4	75.7–109.9	88.0 ± 4.6	11.0	12.6	62.3–87.8	71.2 ± 2.3	3.5	7.7
Dryopteridaceae												
Cyclodium meniscioides var. *meniscioides*	25.2–34.3	30.3 ± 1.1	2.7	8.8	40.7–60.7	46.6 ± 1.9	4.8	10.3	25.9–40.0	28.5 ± 1.2	3.0	10.4
Polypodiaceae												
Campyloneurum phyllitidis	24.2–38.4	25.9 ± 1.2	2.8	11.0	36.0–47.5	43.8 ± 1.2	3.1	7.1	19.1–31.8	26.9 ± 1.1	2.6	9.7
Polypodium fraxinifolium	25.5–38.4	31.0 ± 1.0	2.4	7.9	43.5–56.5	50.5 ± 1.5	3.7	7.3	20.7–31.4	26.1 ± 1.3	3.0	11.7
P. ptilodon	31.7–38.9	34.6 ± 0.8	1.2	5.4	43.8–58.5	51.0 ± 1.6	3.9	7.6	29.5–37.5	33.2 ± 1.0	2.3	7.0
Thelypteridaceae												
Thelypteris chrisodioides	29.3–36.0	32.3 ± 0.9	2.2	7.0	44.6–56.2	49.4 ± 1.2	2.9	5.9	31.2–37.6	33.8 ± 0.7	1.6	4.8
T. dentata	31.6–45.3	39.2 ± 1.2	2.8	7.3	49.4–72.6	56.9 ± 1.8	4.4	7.8	35.4–46.4	40.4 ± 1.1	2.7	6.7
T. dutrai	24.4–29.3	26.9 ± 0.6	1.4	5.2	39.9–47.1	43.8 ± 0.8	1.9	4.5	24.1–34.0	27.3 ± 2.3	3.1	11.3
T. interrupta	33.4–42.7	37.9 ± 1.1	2.6	6.9	44.3–58.4	53.5 ± 2.0	3.8	7.1	29.5–41.9	32.2 ± 1.7	3.4	9.4
T. longifolia	37.8–45.1	41.1 ± 5.6	3.5	8.5	50.3–59.2	54.6 ± 2.2	2.6	4.8	37.0–45.6	39.3 ± 2.4	2.8	7.2
T. rivularioides	33.5–42.7	40.4 ± 1.8	3.5	6.6	48.6–60.5	53.4 ± 2.3	2.6	6.3	43.9–44.4	37.7 ± 0.9	2.3	6.0
T. serrata	24.4–33.0	28.2 ± 1.0	2.5	8.9	37.0–47.4	43.2 ± 1.1	2.7	6.3	24.2–32.3	28.2 ± 1.1	2.7	9.5

TABLE 4. Measurements of length and margin of scar of monolete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu. Dashes (—) = not measured.

Species	Scar length				Scar margin			
	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)	S (μm)	V.C. (%)
Aspleniaceae								
Asplenium serra	16.0–30.5	21.6 ± 1.2	2.9	13.7	—	—	—	—
Blechnaceae								
Blechnum brasiliense	15.1–33.4	24.6 ± 1.6	3.8	15.6	—	—	—	—
B. glandulosum	14.6–24.2	20.3 ± 0.9	2.3	11.5	—	—	—	—
B. raddianum	36.1–41.4	37.4 ± 1.1	1.6	4.3	4.2–7.2	6.2 ± 0.8	1.1	17.8
B. serrulatum	17.5–30.2	22.8 ± 2.8	3.9	17.4	—	—	—	—
Salpichlaena volubilis	39.1–48.3	44.4 ± 1.2	3.0	6.8	4.5–7.7	6.1 ± 0.8	1.1	17.4
Dryopteridaceae								
Cyclodium meniscioides var. *meniscioides*	23.6–36.4	29.1 ± 1.5	3.6	10.9	—	—	—	—
Polypodiaceae								
Campyloneurum phyllitidis	22.9–32.8	27.8 ± 1.2	3.0	10.9	—	—	—	—
Polypodium fraxinifolium	26.6–38.8	31.1 ± 1.4	3.3	10.8	—	—	—	—
P. ptilodon	18.7–33.2	27.5 ± 1.7	2.9	10.6	3.6–6.6	4.8 ± 0.8	1.1	24.1
Thelypteridaceae								
Thelypteris chrisodioides	25.0–34.8	31.2 ± 1.3	3.2	10.2	2.2–4.6	3.8 ± 0.5	0.8	20.0
T. dentata	28.1–32.8	30.8 ± 3.2	2.0	6.6	1.6–2.1	1.9 ± 3.3	0.4	19.6
T. dutrai	20.9–25.9	23.5 ± 1.7	1.6	6.9	1.0–1.1	1.1 ± 0.3	0.1	3.0
T. interrupta	26.5–41.9	32.2 ± 1.9	3.8	11.8	0.9–2.0	1.7 ± 0.2	0.3	19.4
T. longifolia	25.8–35.2	31.1 ± 3.4	3.2	10.5	—	—	—	—
T. rivularioides	—	—	—	—	—	—	—	—
T. serrata	16.8–26.5	22.9 ± 1.3	2.4	10.6	—	—	—	—

TABLE 5. Measurements of exospore of monolete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu. Dashes (—) = not measured.

Species	Sexine		External nexine	
	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)
Aspleniaceae				
Asplenium serra	—	—	—	—
Blechnaceae				
Blechnum brasiliense	0.4–0.6	0.5 ± 0.1	0.8–1.0	0.9 ± 0.1
B. glandulosum	0.7–1.1	0.9 ± 0.2	0.9–1.6	1.2 ± 0.1
B. raddianum	0.7–1.0	0.9 ± 0.1	1.1–1.3	1.2 ± 0.1
B. serrulatum	0.5–0.6	0.5 ± 0.1	0.6–0.7	0.6 ± 0.1
Salpichlaena volubilis	0.5–0.8	0.7 ± 0.1	0.8–1.8	1.2 ± 0.2
Dryopteridaceae				
Cyclodium meniscioides var. *meniscioides*	—	—	—	—
Polypodiaceae				
Campyloneurum phyllitidis	0.5–0.7	0.6 ± 0.1	0.7–0.9	0.8 ± 0.1
Polypodium fraxinifolium	3.5–4.4	4.0 ± 0.2	0.6–1.3	1.0 ± 0.1
P. ptilodon	1.9–2.5	2.3 ± 0.2	0.7–1.5	1.1 ± 0.2
Thelypteridaceae				
Thelypteris chrisodioides	1.4–2.3	1.7 ± 0.2	0.8–1.2	0.9 ± 0.1
T. dentata	—	—	—	—
T. dutrai	0.9–1.2	1.0 ± 0.1	0.6–0.9	0.7 ± 0.1
T. interrupta	0.7–1.0	0.9 ± 0.1	1.0–1.3	1.2 ± 0.1
T. longifolia	—	—	—	—
T. rivularioides	0.9–1.8	1.3 ± 0.2	0.6–1.0	0.7 ± 0.1
T. serrata	—	—	—	—

The perispore of *Cyathea delgadii* was observed only in immature spores and in this case it could not be removed by 2 min of acetolysis. Perispore was not observed in spores of *C. phalerata* after acetolysis.

The perispore of *Lindsaea lancea* was easily removed by acetolysis. The concave areas between the arms of the scar are typical of the genus *Lindsaea* (Tryon and Tryon, 1982). The measurements presented in the present work agree with Erdtman and Sorsa (1971), but they described the exospore as psilate.

Dicranopteris includes both species with trilete and with monolete spores (Tryon and Lugardon, 1991).

The perispore was not observed in acetolysed spores of *Trichomanes cristatum* and *T. emarginatum*.

It was not possible to measure the exospore of *Lycopodiella cernua* because the depression around the scar obscured its dimensions.

Perispore was only present in immature spores of *Osmunda cinnamomea* and *O. regalis*. In general, the spores of these two species were very fragile. The description of *O. cinnamomea* agrees with the one presented by Erdtman

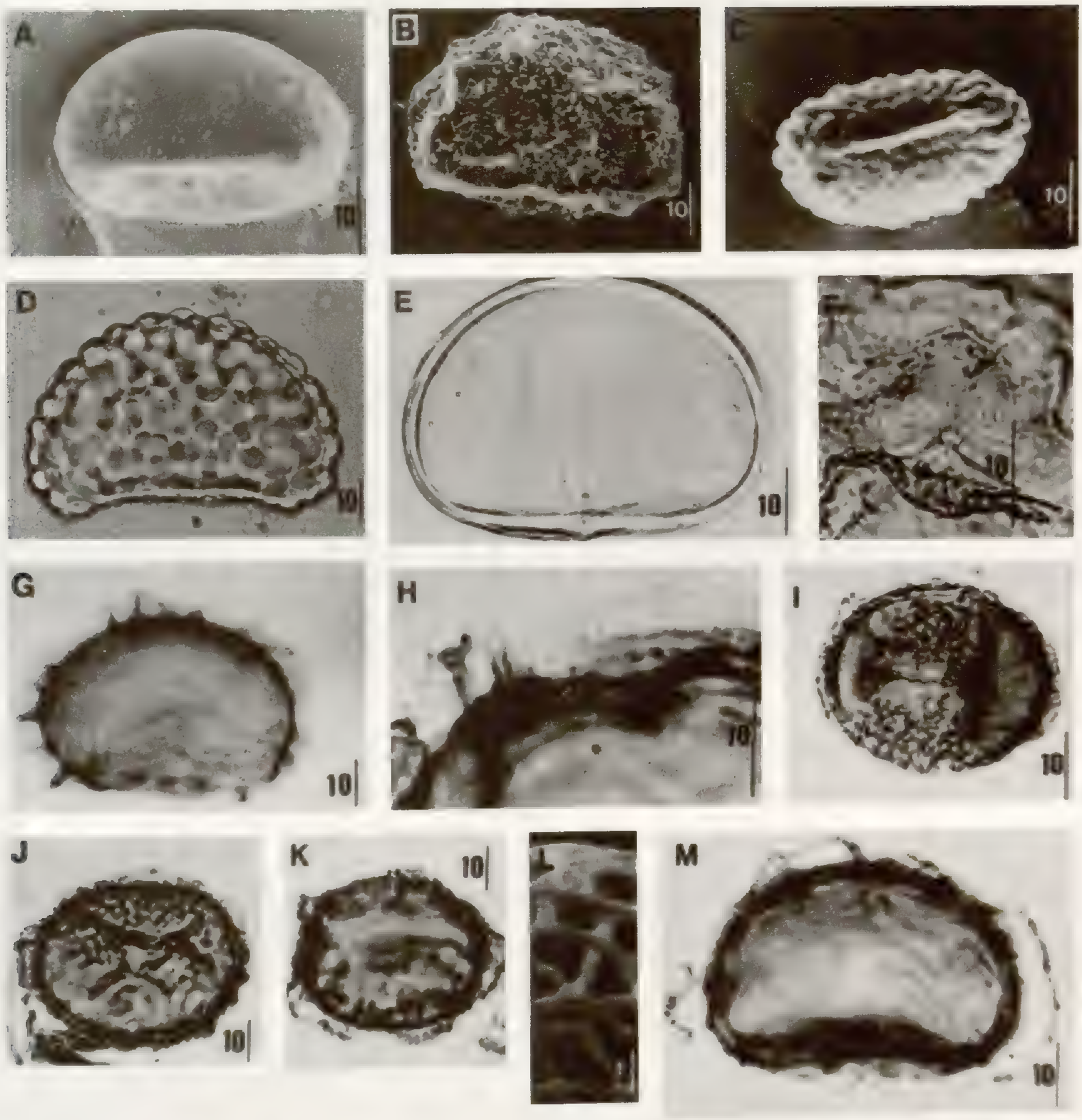


FIG. 3. Monolete spores in the study. A) *Blechnum glandulosum*, equatorial view (SM). B) *B. raddianum*, equatorial view (SM). C) *Polypodium fraxinifolium*, proximal polar view (SM). D) *P. ptilodum*, equatorial view (OM). E) *Thelypteris chrisodioides*, spore without perispore, equatorial view (OM). F) *T. chrisodioides*, detail of perispore ornamentation (OM). G) *T. dentata*, spore with perispore, equatorial view (OM). H) *T. dentata*, detail of perispore ornamentation (OM). I) *T. dutrai*, spore with perispore, proximal polar view (OM). J) *T. interrupta*, spore with perispore, proximal polar view (OM). K) *T. longifolia*, spore with perispore, proximal polar view (OM). L) *T. rivularioides*, detail of perispore ornamentation (SM). M) *T. serrata*, equatorial view (OM). M = optical microscopy; SM = scanning electronic microscopy.

and Sorsa (1971), the spores being only larger in size. The measurements of spores of *O. regalis* are very similar to the ones presented by Erdtman and Sorsa (1971).

The description and measurements of spores of *Pityrogramma calomelanos* are similar to those presented by Nayar and Devi (1967), de la Sota (1972), Panigrahi (1976), and Tryon and Lugardon (1991). In *P. trifoliata*, spores with and without perispore were observed after 2 min of acetolysis. The ornamen-

TABLE 6. Morphology of trilete fern spores. Abbreviations: E = equatorial view; OM = optical microscopy; Ex = Exine; Pe = perine; SM = scanning electron microscopy.

Species	Size	Shape	Ornamentation
Cyatheaceae			
Cyathea delgadii	medium	tetrahedral-globose	Pe: tenuous and translucid Ex: verrucate; rodlets over and between the verrucae observed in SM; three layers Scar: developed margin
C. phalerata (Fig. 4A)	large	tetrahedral-globose	Pe: absent Ex: granulate; two layers Scar: developed margin
Dennstaedtiaceae			
Lindsaea lancea	medium	tetrahedral-globose, concave areas between the arms of the scar	Pe: with rodlets (under SM) Ex: granulate and with fissures; two layers Scar: developed margin
L. quadrangularis (Fig. 4B)	medium	tetrahedral-globose, concave areas between the arms of the scar	Pe: tenuous, granulate, presenting globules under the SM Ex: psilate; two layers
Pteridium aquilinum	medium	tetrahedral-globose, concave areas between the arms of the scar	Pe: granulate Ex: surface slightly undulate; two layers Scar: developed margin
Gleicheniaceae			
Dicranopteris flexuosa	medium	tetrahedral	Pe: granular deposits (SM) Ex: granulate; two layers
Hymenophyllaceae			
Trichomanes cristatum (Fig. 4C, 4D)	medium	spheroidal	Pe: absent Ex: papillate, baculate or echinate; two layers
T. emarginatum (Fig. 4E)	medium	spheroidal	Pe: absent Ex: granulate; two layers Scar: developed margin

TABLE 6. Continued.

Species	Size	Shape	Ornamentation
Lycopodiaceae			
Lycopodiella cernua	medium	spheroidal to tetrahedral-globose, with concave areas near the scar	P: thin; granulate; with some globules Ex: granulate on the proximal face and partially anastomosed low folds on the distal face; two layers
Osmundaceae			
Osmunda cinnamomea	large	spheroidal	Pe: tenuous; translucid Ex: two types of spore ornamentation: (a) pilate and baculate and (b) rugulate (this is the most common in spores acetolysed for 2 minutes); three layers Scar: developed margin
O. regalis L.	large	spheroidal	Pe: tenuous Ex: tuberculate; tubercles overlaid with sparse echinae; three layers Scar: developed margin
Pteridaceae			
Adiantum fructuosum (Fig. 4F)	medium	tetrahedral-globose	Pe: thin; most areas with scale-like structures Ex: granulate; two layers Scar: developed margin
Pityrogramma calomelanos	medium	tetrahedral-globose, the distal face sub-hemispherical	Pe: thin; granulate Ex: surface coarsely rugate on the proximal face and reticulate on the distal face, these two types are separated by a prominent equatorial flange (three ridges); two layers Scar: developed margin
P. trifoliata	medium	tetrahedral-globose, the distal face sometimes convex	Pe: tenuous; granulate; dark-brow in acetolised spores Ex: verrucate on the proximal face and psilate on the distal face; there is no equatorial flange; two layers Scar: developed margin

TABLE 7. Measurements of polar and equatorial axes of trilete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu. Dashes (—) = not measured.

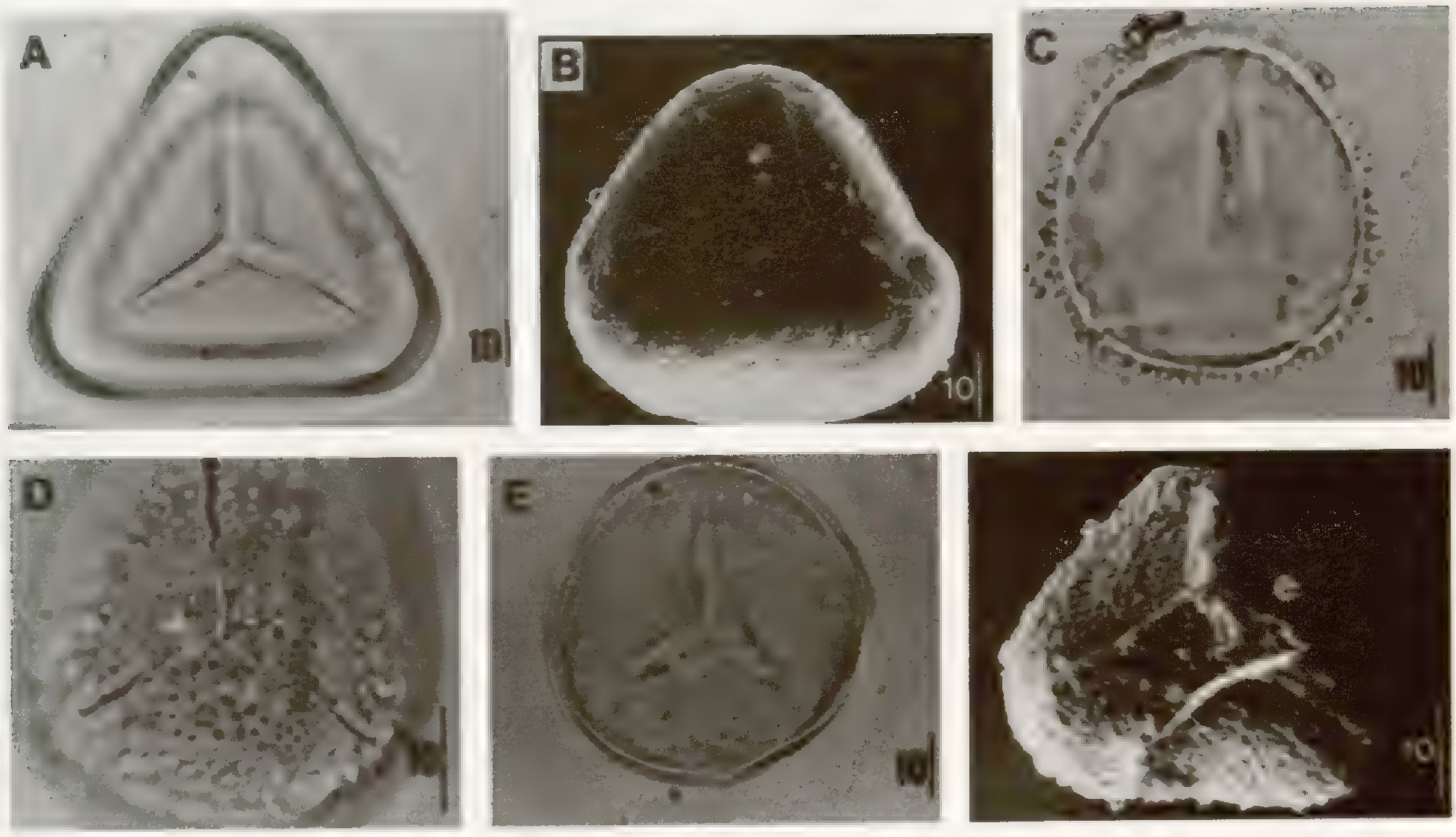
Species	Polar axis				Equatorial axis			
	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\bar{x}$ (μm)	S (μm)	V.C. (%)
Cyatheaceae								
Cyathea delgadii	33.0–42.7	36.0 ± 0.9	2.3	6.4	42.4–53.0	47.7 ± 1.2	2.9	6.0
Cyathea phalerata	41.8–51.6	47.2 ± 1.2	2.9	6.2	56.2–63.1	60.8 ± 0.8	1.9	3.1
Dennstadtiaceae								
Lindsaea lancea	21.3–27.6	24.5 ± 0.7	1.7	7.0	26.3–32.6	28.8 ± 0.7	1.6	5.7
Lindsaea quadrangularis	19.5–28.0	24.1 ± 0.8	2.1	8.5	26.8–33.0	30.7 ± 1.0	2.0	6.4
Pteridium aquilinum var. *arachnoideum*	22.1–28.9	25.8 ± 1.5	2.1	7.9	27.7–37.6	33.3 ± 3.2	3.9	11.7
Gleicheniaceae								
Dicranopteris flexuosa	20.1–37.3	30.3 ± 1.6	3.9	12.7	37.0–48.3	41.9 ± 1.1	2.7	6.5
Hymenophyllaceae								
Trichomanes cristatum	25.0–34.7	29.7 ± 0.9	2.1	7.2	35.7–48.3	39.1 ± 0.8	2.0	5.0
T. emarginatum	32.9–41.0	36.5 ± 0.8	1.9	5.4	41.9–47.0	43.9 ± 0.9	2.7	6.2
Lycopodiaceae								
Lycopodiella cernua	17.9–25.8	20.5 ± 0.8	1.9	9.2	28.7–31.8	30.9 ± 0.9	2.1	6.8
Osmundaceae								
Osmunda cinnamomea	44.9–57.8	53.5 ± 1.5	3.6	6.7	50.0–71.7	62.0 ± 2.6	5.3	8.6
O. regalis	46.8–72.4	56.4 ± 2.3	5.6	9.9	52.6–72.7	64.6 ± 2.6	6.3	9.7
Pteridaceae								
Adiantum fructuosum	26.1–30.6	29.2 ± 1.2	1.6	5.6	39.1–47.0	43.6 ± 1.8	2.6	5.9
Pityrogramma calomelanos	32.7–48.4	39.1 ± 1.4	3.4	8.7	39.3–54.6	47.0 ± 1.7	4.2	8.9
P. trifoliata	38.5–55.4	43.8 ± 2.3	4.1	9.5	44.8–54.6	50.2 ± 3.8	4.6	9.2

TABLE 8. Measurements of length, arm, and margin of scar of trilete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu. Dashes (—) = not measured.

Species	Scar length				Scar arm				Scar margin			
	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	S (μm)	V.C. (%)
Cyatheaceae												
Cyathea delgadii	28.0–37.3	33.6 ± 1.3	3.1	9.3	12.1–21.2	16.6 ± 1.1	2.6	15.5	1.8–5.0	3.2 ± 0.7	0.9	28.6
Cyathea phalerata	30.9–45.1	38.8 ± 1.5	3.7	9.6	17.4–28.0	22.7 ± 1.0	2.4	10.7	2.6–4.8	3.7 ± 0.3	0.6	17.4
Dennstadtiaceae												
Lindsaea lancea	16.8–25.8	20.0 ± 0.9	2.0	9.8	8.6–14.2	11.6 ± 0.6	1.4	11.7	0.8–1.5	1.0 ± 0.1	0.2	16.4
Lindsaea quadrangularis	16.7–23.6	19.7 ± 1.0	1.9	9.9	10.1–14.7	11.9 ± 0.7	1.3	11.4	—	—	—	—
Pteridium aquilinum var. *arachnoideum*	19.2–26.2	22.7 ± 2.2	2.7	11.8	8.3–14.5	12.6 ± 1.5	1.8	14.6	1.3–2.0	1.6 ± 0.9	0.4	23.4
Gleicheniaceae												
Dicranopteris flexuosa	25.3–36.3	29.2 ± 1.3	3.1	10.6	11.7–21.4	17.1 ± 0.9	2.4	13.9	—	—	—	—
Hymenophyllaceae												
Trichomanes cristatum	22.0–34.2	27.7 ± 1.0	2.4	8.8	10.7–19.0	15.4 ± 0.8	2.0	13.0	—	—	—	—
T. emarginatum	21.0–29.7	24.3 ± 1.8	4.7	19.5	10.2–15.1	12.5 ± 0.1	2.5	19.8	1.3–1.9	1.6 ± 0.9	0.4	23.4
Lycopodiaceae												
Lycopodiella cernua	15.1–20.1	17.3 ± 0.6	1.6	8.9	8.7–14.4	11.1 ± 0.5	1.2	10.5	—	—	—	—
Osmundaceae												
Osmunda cinnamomea	31.2–48.1	40.3 ± 1.8	4.4	10.9	17.4–26.7	21.9 ± 1.8	2.6	11.8	3.0–5.3	3.9 ± 0.4	0.6	15.3
O. regalis	24.8–44.0	38.8 ± 2.1	5.0	12.9	12.9–33.2	22.9 ± 1.8	4.4	19.0	1.9–3.6	2.7 ± 0.4	0.6	23.6
Pteridaceae												
Adiantum fructuosum	26.6–35.4	32.1 ± 2.0	2.9	9.1	9.9–20.4	16.9 ± 2.3	3.2	19.1	1.7–1.9	1.8 ± 0.1	0.1	5.3
Pityrogramma calomelanos	21.2–32.4	26.6 ± 1.3	3.3	12.3	13.4–21.9	17.4 ± 0.9	2.2	12.5	1.4–1.8	1.7 ± 0.1	0.1	8.5
P. trifoliata	26.3–38.7	31.8 ± 3.9	4.7	14.9	14.9–21.2	18.4 ± 1.8	2.2	11.9	0.9–1.0	1.0 ± 0.1	0.1	5.0

TABLE 9. Measurements of exospore of trilete spores of pteridophyta from the gallery forest of the Reserva Biológica e Estação Experimental de Moji Guaçu. Dashes (—) = not measured.

Species	Sexine		External nexine		Internal nexine	
	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)	Range (μm)	$\bar{x} \pm S\,\bar{x}$ (μm)
Cyatheaceae						
Cyathea delgadii	1.0–3.1	1.7 ± 0.6	0.3–0.6	0.5 ± 0.1	0.9–1.8	1.2 ± 0.2
Cyathea phalerata	1.2–1.4	1.3 ± 0.1	0.9–1.4	1.2 ± 0.1	—	—
Dennstadtiaceae						
Lindsaea lancea	0.8–1.1	0.9 ± 0.1	0.6–1.0	0.9 ± 0.1	—	—
Lindsaea quadrangularis	1.1–1.7	1.3 ± 0.2	0.5–0.8	0.7 ± 0.1	—	—
Pteridium aquilinum var. *arachnoideum*	0.6–0.9	0.7 ± 0.1	0.9–1.3	1.0 ± 0.3	—	—
Gleicheniaceae						
Dicranopteris flexuosa	0.6–0.9	0.8 ± 0.1	0.9–1.2	1.1 ± 0.1	—	—
Hymenophyllaceae						
Trichomanes cristatum	0.9–2.3	1.8 ± 0.3	0.7–1.3	0.9 ± 0.1	—	—
T. emarginatum	0.6–0.9	0.7 ± 0.1	0.6–0.9	0.8 ± 0.1	—	—
Lycopodiaceae						
Lycopodiella cernua	—	—	—	—	—	—
Osmundaceae						
Osmunda cinnamomea	2.0–2.6	2.2 ± 0.2	0.4–0.8	0.5 ± 0.1	0.7–1.2	0.9 ± 0.1
O. regalis	1.4–3.8	2.2 ± 0.5	0.4–1.2	0.7 ± 0.2	0.6–1.6	1.0 ± 0.2
Pteridaceae						
Adiantum fructuosum	0.8–0.9	0.8 ± 0.1	0.8–0.9	0.9 ± 0.1	—	—
Pityrogramma calomelanos	—	—	—	—	—	—
P. trifoliata	0.7–0.9	0.8 ± 0.1	0.9–1.0	0.9 ± 0.1	—	—



FIGS. 4. Trilete spores in the study. A) *Cyathea phalerata*, proximal polar view (OM). B) *Lindsaea quadrangularis*, proximal polar view (SM). C) *Trichomanes cristatum*, spore without perispore: proximal polar view (OM). D) *T. cristatum*, detail of exospore ornamentation. E) *T. emarginatum*, proximal polar view (OM). F) *Adiantum fructuosum*, proximal polar view (SM); e = scale-like structure. OM = optical microscopy; SM = scanning electronic microscopy

tation of *P. trifoliata* does not agree with the description by Tryon and Lugardon (1991), who noted a granulate surface.

SPORE RAIN.—It was impossible to separate some of the species of pteridophytes from the spores found in the spore rain, because their spore characteristics are very similar. Thus, the following spore types represent potential mixtures of indistinguishable species: type *Lindsaea*, type *Campyloneurum phyllitidis*/*Pleopeltis angusta*, type *Thelypteris* (perispore with folds), and type *Thelypteris*/*Asplenium* (reticulate perispore).

In general, the highest percentage of spores was found in the collector located in the marsh and the lowest in the open cerrado (Fig. 5). At all four localities, the highest percentage of spores was observed in January and February (summer). The zero values in Fig. 5 (with the exception of February 1994, when no sampling was done) represent absence of spores of pteridophyta in those samples, whereas pollen grains were present in every month of the year. There is a positive correlation between the higher number of spores and the peaks of high temperature and rain during the period of the experiment in the Reserve (Fig. 2). A similar correlation also was found by Hanumantha et al. (1991) in the Chikamagalur region of Karnataka, India; the higher concentration of spores occurred in the period of high humidity and the lower in the dryer months of the year. On the other hand, Silva (1989) showed a negative correlation between spores and rain and a positive correlation with temperature in Portugal.

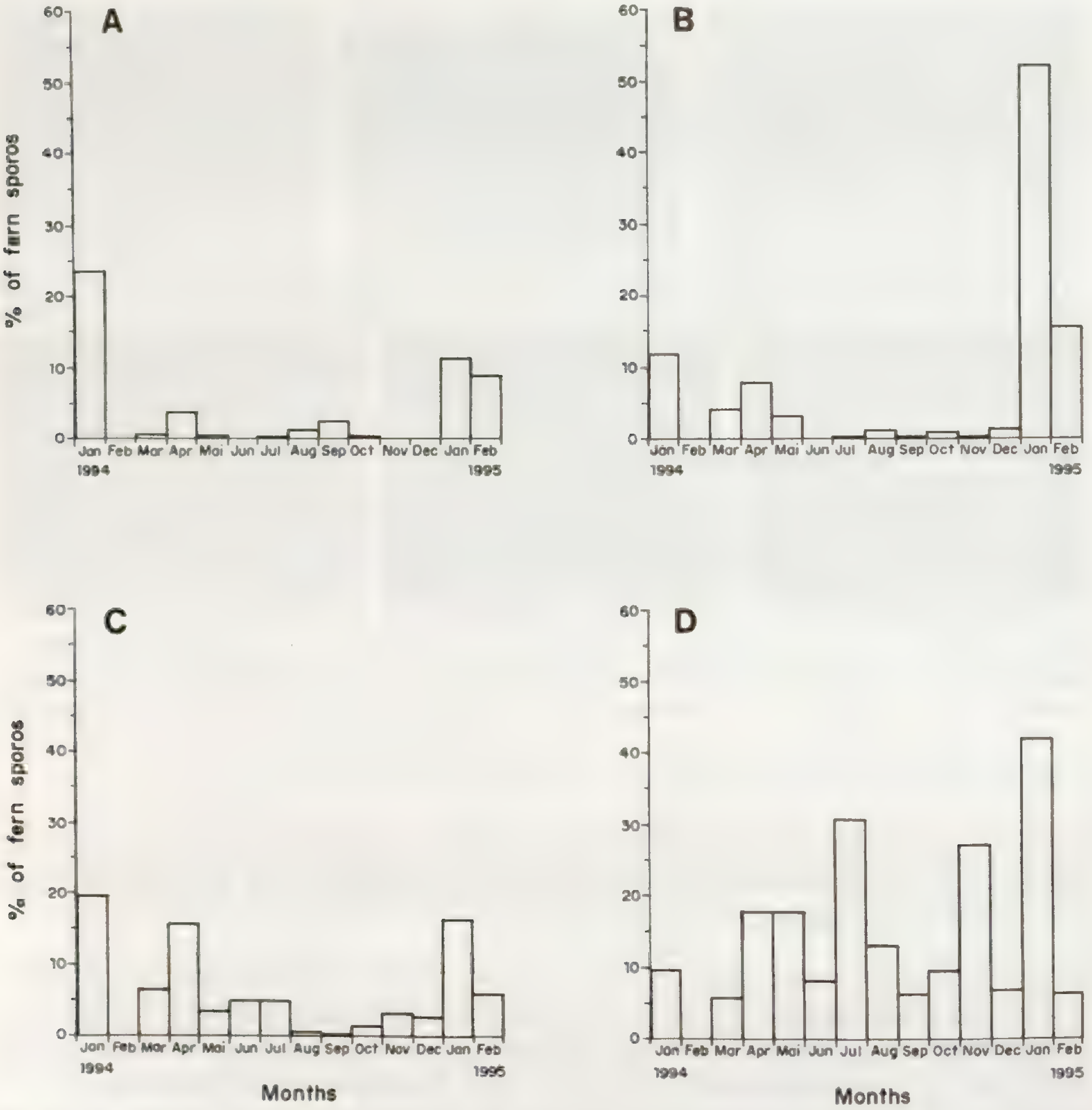


FIG. 5. Percentage of spores of pteridophyta in relation to the total number of grains (pollen + spores) in the spore rain of Reserva Biológica e Estação Experimental de Moji Guaçu, between January 1994 and February 1995. A) Open cerrado. B) Cerrado. C) Gallery forest. D) Marsh.

Spores of 16 species/types were present in the spore rain. Only six were present in all four localities: *Blechnum glandulosum, Cyathea delgadii, Osmunda regalis, Polypodium ptilodon, Thelypteris* type, and *Campyloneurum phyllitidis/Pleopeltis angusta* type. The distribution of the spores by habitat is shown in Table 10. The lowest diversity of species was found in cerrado, with only six species/types, and the highest in gallery forest with 14 species/types. The six species/types found in the cerrado were also found in the other three localities. Spores of *Dicranopteris flexuosa* and *Polypodium latipes* were found only in the open cerrado. All the species/types found in the marsh were also found in the gallery forest.

TABLE 10. Distribution of spores of species/types of pteridophyta in open cerrado, cerrado, gallery forest and marsh, from January 1994 to February 1995, in the spore rain of Reserva Biológica e Estação Experimental de Moji Guaçu (SP).

	Open cerrado	Cerrado	Gallery forest	Marsh
Blechnum brasiliense			×	×
Blechnum glandulosum	×	×	×	×
Salpichlaena volubilis	×		×	
Cyathea delgadii	×	×	×	×
Cyathea phalerata	×		×	
Lindsaea			×	×
Dicranopteris flexuosa	×			
Lycopodiella cernua			×	×
Osmunda cinnamomea			×	
Osmunda regalis	×	×	×	×
Campyloneurum phyllitidis/Pleopeltis angusta	×	×	×	×
Polypodium fraxinifolium			×	
Polypodium latipes	×			
Polypodium ptilodon	×	×	×	×
Thelypteris	×	×	×	×
Thelypteris/Asplenium serra	×		×	×

The distribution of each species/type in each month of the experimental period in open cerrado, cerrado, gallery forest, and marsh is presented in Table 11. This table also shows that the highest diversity of species/types occurred in January 1995 (11 types) and March 1994 (10 types); only 2 types (*Blechnum glandulosum* and *Cyathea delgadii)* were found in October 1994. The number of spores/type differed in January 1994 and 1995. According to Chen and Chien (1986), both the quality and the quantity of spore and pollen grains in the air change from year to year in the same area because of weather conditions and the phenological behavior of plants.

The spores of *Cyathea delgadii* were present throughout the year at all four localities and were more numerous than those of any other species. It was estimated by us that one leaf of this species produces 269 million of spores, a median value if compared with the ones calculated by Page (1979). Also, in the Reserve this species produces spores year-round with a peak in January–March (during some years the fronds are destroyed by frost during the winter). The presence of *Cyathea delgadii* in the gallery forest only and the appearance of its spores at all four localities studied show that this species is very efficient in the dispersal of its spores, but that there apparently is some difficulty with establishment of sporophytes in some habitats. Pickrill and Fenner (1992) show that spores of tree ferns are dispersed for short distances, but they are produced in large amounts, as is the case of *Cyathea delgadii.*

In each sample, 500 total grains (pollen and spores) were counted. Thus, from Fig. 5 it is possible to develop an idea of the numbers of spores in 500 grains from the pollen/spore rain. The highest percent of fern spores on Fig.

TABLE 11. Percentage of spores of each species/type of pteridophyta in relation to the total number of pteridophyta, in the spore rain of Reserva Biológica e Estação Experimental de Moji Guaçu (SP), from January 1994 to February 1995. Localities: A) open cerrado; B) cerrado; C) gallery forest; D) marsh.

Species/type	Locality	% of spores of each species/type in relation to total number of spores of pteridophytes												
		Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Blechnum brasiliense	C	0	5.71	0	12.50	0	0	0	0	0	55.55	60.00	0	53.11
	D	0	0	0	0	0	0	0	0	0	0	4.88	0.90	0
Blechnum glandulosum	A	31.82	66.67	8.33	0	0	0	33.33	100.00	0	0	0	2.00	0
	B	33.33	0	0	0	0	0	66.67	0	100.00	0	0	0.36	8.19
	C	8.16	0	25.00	18.75	0	3.85	20.00	0	0	0	0	10.00	0
	D	4.41	16.13	0	0	0	0	0	0	0	0	0	0.46	0
Salpichlaena volubilis	A	0	0	0	0	0	0	33.33	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	32.22	6.25
Cyathea delgadii	A	13.64	33.33	91.67	0	0	100.00	0	0	100.00	0	0	86.00	15.00
	B	27.78	50.00	100.00	100.00	0	100.00	33.33	0	0	0	100.00	98.23	83.61
	C	59.18	51.43	62.50	56.25	82.76	92.30	80.00	100.00	100.00	5.56	13.33	46.67	21.88
	D	95.59	77.43	96.30	95.60	92.86	95.11	97.92	50.00	100.00	0	85.36	94.98	91.43
Cyathea phalerata	A	0	0	0	0	0	0	0	0	0	0	0	2.00	0
	C	0	11.43	0	0	10.34	0	0	0	0	0	0	1.11	0
Lindsaea	C	0	5.71	0	0	0	0	0	0	0	0	6.66	0	0
	D	0	3.22	0	0	0	0	0	0	0	0	0	0	0
Dicranopteris flexuosa	A	0	0	0	0	0	0	16.97	0	0	0	0	0	0
Lycopodiella cernua	C	0	0	0	0	3.45	0	0	0	0	0	0	0	0
	D	0	3.22	0	0	0	0	0	0	0	0	2.44	0	0
Osmunda cinnamomea	C	0	8.58	0	0	0	0	0	0	0	0	0	0	0
Osmunda regalis	A	4.54	0	0	0	0	0	16.67	0	0	0	0	0	0
	B	5.55	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	8.57	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	1.85	0	7.14	4.35	2.08	0	0	0	0	0.46	2.85
Campyloneurum phyllitis/ Pleopeltis angusta	A	0	0	0	50	0	0	0	0	0	0	0	0	15.00
	B	0	0	0	0	0	0	0	0	0	0	0	0	6.56
	C	0	0	0	0	0	0	0	0	0	16.67	6.66	1.11	3.13
	D	0	0	0	0	0	0	0	0	0	0	4.88	0	2.86
Polypodium fraxinifolium	C	0	2.86	0	6.25	0	0	0	0	0	5.55	0	3.33	3.13

TABLE 11. Continued.

Species/type	Locality	% of spores of each species/type in relation to total number of spores of pteridophytes												
		Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Polypodium latipes	A	0	0	0	50	0	0	0	0	0	0	0	0	70.00
Polypodium ptilodon	A	50.00	0	0	0	0	0	0	0	0	0	0	2.00	0
	B	16.67	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	3.85	0	0	0	0	0	0	0
	D	0	0	0	0	0	0.54	0	0	0	0	0	0	0
Thelypteris	A	0	0	0	0	0	0	0	0	0	0	0	6.00	0
	B	16.67	50.00	0	0	0	0	0	0	0	0	0	1.41	1.64
	C	26.53	5.71	6.25	6.25	3.45	0	0	0	0	16.67	6.67	5.56	12.50
	D	0	0	1.85	4.40	0	0	0	0	0	0	2.44	2.74	2.86
Thelypteris/Asplenium serra	A	0	0	0	0	0	0	0	0	0	0	0	2.00	0
	B	0	0	6.25	0	0	0	0	0	0	0	6.67	0	0
	C	0	0	6.25	0	0	0	0	0	0	0	6.67	0	0
	D	0	0	0	0	0	0	0	50	0	0	0	0.46	0

5 is in the cerrado sample for January 1995. The number of fern spores for this peak is 260 (52%). From data in Table 11, this value can be further divided into 254 spores of *Cyathea delgadii* and 6 spores of *Thelypteris* type.

ACKNOWLEDGMENTS

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American Fern Journal 88(3):138–142 (1998)

Fungal Associations with *Isoetes* Species

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ABSTRACT.—The fungal associations present in the roots and corms of four species of *Isoetes* found in Rajasthan, India, are described. Fungal fruiting bodies and chlamydospores are assigned to the mycorrhizal genus *Glomus,* and the septate branched intracellular mycelium is assigned to the genus *Rhizoctonia* of the Deuteromycotina.

Mycorrhizal associations in angiosperms are very well known in the literature (Gerdemann, 1965; Mosse, 1973; Schenck and Perez, 1988; Ammani et al., 1994; Gupta and Mukherji, 1996), but there are few references to mycorrhizae in pteridophytes (Nair and Mahabale, 1975; Harsh and Sharma, 1995), and probably none on the genus *Isoetes* L. (Isoetaceae). In the present paper, fungal associations are described in the roots and corms of terrestrial and aquatic species of *Isoetes* found in Rajasthan, India (Gena and Bhardwaja, 1984; Sharma et al., 1985; Sharma, 1989; Sharma and Harsh, 1993).

MATERIALS AND METHODS

All four species of *Isoetes* in Rajasthan, i.e., *I. coromandelina* L.f., *I. rajasthanensis* Gena & Bhardwaja, *I. reticulata* Gena & Bhardwaja, and *I. tuberculata* Gena & Bhardwaja (Gena and Bhardwaja, 1984; Sharma, 1989), were examined in this study. *Isoetes tuberculata* is aquatic, whereas all others are amphibious or terrestrial. Specimens were fixed in FAA and microtome sections at 8–10 μm were cut through corms in various planes following dehydration and imbedding. Sections were stained with a combination of safranin and haematoxylon (Johansen, 1940). Slides were mounted in dilute Canada-balsam. Sections were viewed using light microscopy and recorded photographically.

RESULTS

In roots of *Isoetes,* the fungus has a septate and branched mycelium, and forms arbuscules like bunches of hyphae in cortical cells. In corms of *Isoetes,* scattered chlamydospores or fruiting bodies, each containing a number of "spores," are present. The mycelium observed in roots of *Isoetes* is related to the genus *Rhizoctonia* DC. ex Fr. of the Deuteromycotina (Alexopoulos and Mims, 1979; Bold et al., 1987). Fruiting bodies in the corms may be related to the vesicular arbuscular mycorrhizal (VAM) genus *Glomus* Tul. & C. Tul. (Schenck and Perez, 1988).

Scales and phyllopodia were present in samples of all four species of *Isoetes* examined in this study (Srivastava and Wagai, 1995). A number of isolated

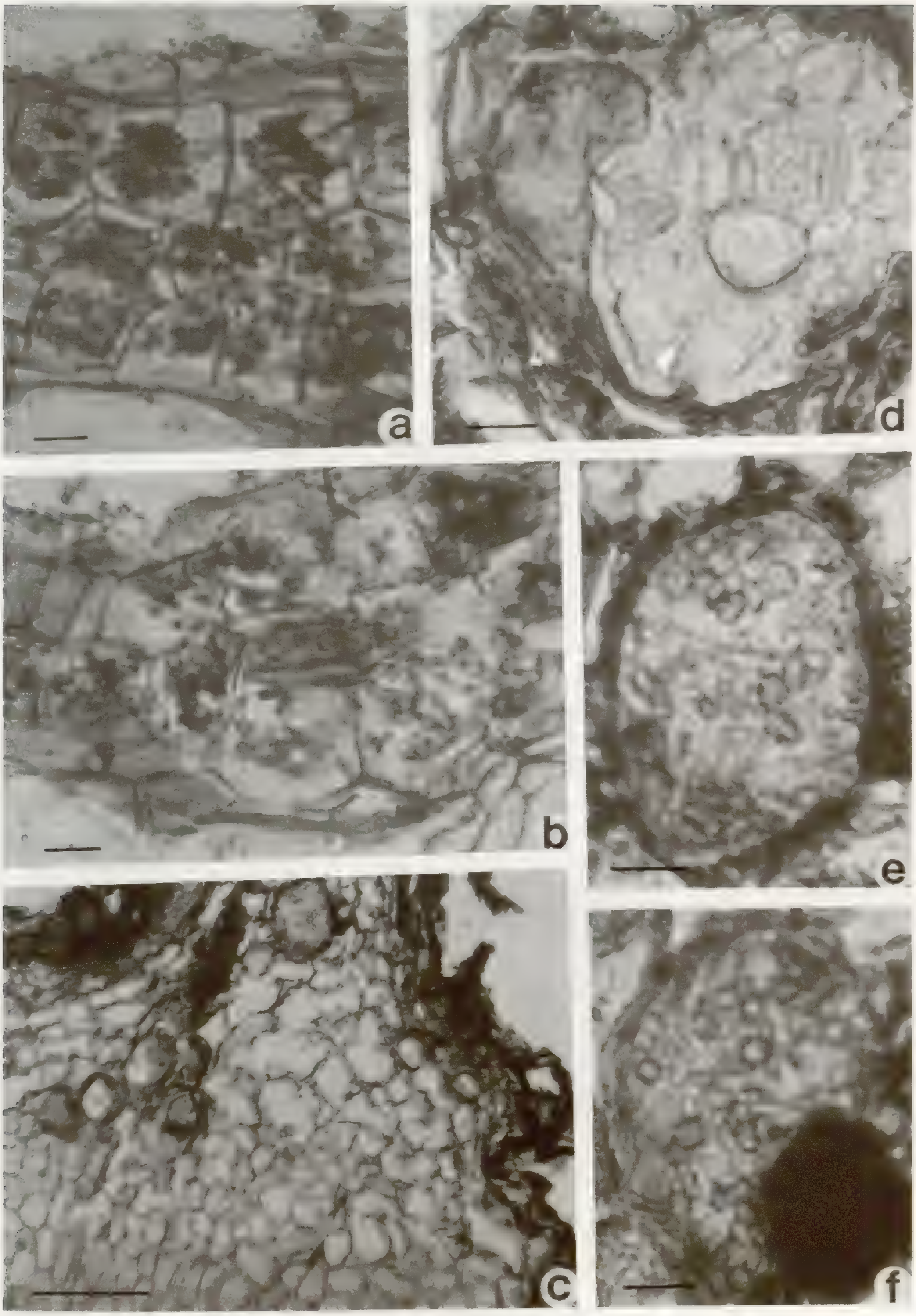

FIG. 1. Anatomical sections of *Isoetes* species. a, b) *I. tuberculata*; a) Longitudinal section of root, the cortical cells with branched and bunched hyphae (arbuscules); b) Oblique cross section of root, the middle cortex cells with branched, septate hyphae. c) *I. rajasthanensis*, cross section of corm, the outer cortex with a fruiting body and chlamydospores. d) *I. reticulata*; cross section of young root with a chlamydospore and balloon-shaped penetrating haustorium. e, f) *I. rajasthanensis*, cross sections of corms with fruiting bodies with hyphae and spores. Scale bars: a, b, d–f = 10 μm; c = 100 μm.

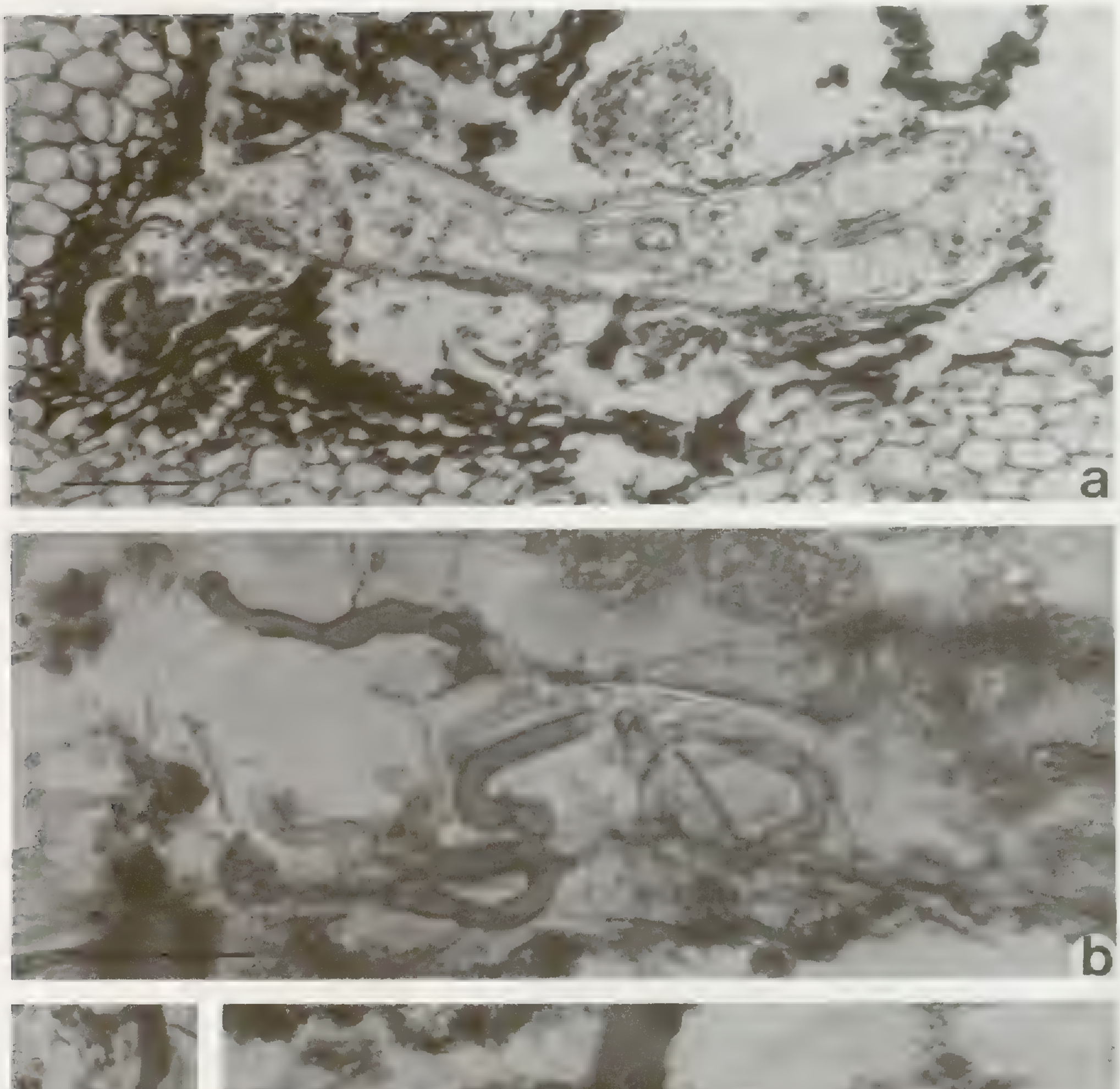

FIG. 2. Anatomical sections of *Isoetes rajasthanensis*. a) Cross and longitudinal sections of roots with fungal mycelia; b, c) Septate and branched intracellular hyphae resembling *Rhizoctonia* sp. in cortical cells of roots; d) Septate hyphae on root surface. Scale bars: a = 100 μm; b–d = 10 μm.

scattered chlamydospores and a few fruiting bodies were observed in the scales and phyllopodia. The globose spores have thin or thick walls and measure 20 × 22 to 30 × 34 μm. In some of the spores, a small portion of the attached mycelium is visible and it appears to be coenocytic and non-septate.

Similar chlamydospores (Fig. 1c) and fruiting bodies (Fig. 1e, f) also are present in the outer cortex in some corms of *I. rajasthanensis* and *I. reticulata*. These fruiting bodies are globular to elongated with well-defined walls. They measure 45 × 50 to 45 × 56 μm and contain either a number of young spores originating from hyphae (Fig. 1e) or spores that are loose and variable in size at maturity (Fig. 1f). The attached mycelium has yet to be seen. The spores are nearly globular with thin or thick walls and range in size from 6 × 8 to 10 × 12 μm. These fruiting bodies may be related tentatively to the common VAM genus *Glomus*.

Identical fungal mycelia were found in roots of both terrestrial and aquatic species collected from widely separated places; e.g., *I. rajasthanensis* from Mt. Abu, *I. reticulata* from Atru, and *I. tuberculata* from Jhalawar. The distance between Mt. Abu and Atru is more than 500 km, and Atru and Jhalawar are more than 150 km apart. Although the soil and climatic conditions are different at Mt. Abu and Atru, the association of an identical fungal mycelium resembling *Rhizoctonia* was observed. This fungus enters the narrow roots from fungal propagules present either between scales and phyllopodia or among roots (Fig. 2a).

In young roots, the vascular bundle is centric (Fig. 1d) and the middle cortex is composed of large parenchyma cells (Fig. 1b). In a mature root, the vascular bundle becomes excentric and the middle cortex develops a large cavity (Bierhorst, 1971; Bold et al., 1987). Within the cortex of roots, the fungus is composed of an irregularly branched and septate mycelium with hyphae ranging from 5–9 μm in diameter (Fig. 2b–d). In many cells of the middle cortex of roots, the mycelium divides repeatedly and forms arbuscules like bunches of intracellular hyphae (Fig. 1a, b).

Reproductive bodies associated with mycelia could not be observed in any of the sections cut through the roots of samples. Interestingly, the shape and size of the cortical cells are not disturbed or deformed by the presence of mycelia and arbuscules in them. This suggests a symbiotic association between the fungus and the cortical cells of the roots. It is hoped that continuing investigations on the study of the rhizosphere of *Isoetes* plants found growing at various places in Rajasthan will result in more exact determinations of the "associated fungi" described in the present paper.

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Shorter Note

A New Station for *Dicranopteris flexuosa* in Franklin County, Florida.—On 7 December 1997, while on a routine field trip to photograph species of Lycopodiaceae, I discovered a new station for *Dicranopteris flexuosa* (Schrad.) L. Underw. (forked fern). The station is located approximately 15.5 air miles north of Apalachicola, Florida (T6S R8W). It consists of a single clump with several fronds and is situated along the upper half of an approximately two foot ditch embankment that is composed of moist sandy soil at the edge of a longleaf and slash pine (*Pinus palustris* Mill., *P. elliottii* Engelm. var. *elliottii*) woodland.

The habitat is similar to that described for the two most recently discovered Florida stations for this species (Burkhalter, Amer. Fern J. 75:79, 1985; Moyroud & Nauman, Amer. Fern J. 79:155, 1989). Associates found within approximately two meters of the specimen include *Agalinis* sp., *Clethra alnifolia* L., *Drosera* cf. *capillaris* Poir., *Gelsemium sempervirens* (L.) J. St.-Hil., *Hypericum cistifolium* Lam., *H. fasciculatum* Lam., *H. microsepalum* (Torr. & A. Gray) A. Gray ex S. Watson, *Lycopodiella prostrata* (R.M. Harper) Cranfill, *Palhinhaea cernua* (L.) Vasc. & Franco, *Pseudolycopodiella caroliniana* (L.) Holub, and *Smilax laurifolia* L. Species found in the edge of the adjacent pineland include *Aristida beyrichiana* Trin. & Rupr., *Centella asiatica* (L.) Urban, *Hypericum brachyphyllum* (Spach) Steud., *H. crux-andreae* (L.) Crantz, *Ilex coriacea* (Pursh) Chapm., *I. glabra* (L.) A. Gray, *I. myrtifolia* Walter, *Lycopodiella alopecuroides* (L.) Cranfill, *Oxypolis ternata* (Nutt.) A. Heller, *Serenoa repens* (Bartram) Small, and *Xyris* sp.

This new station is the sixth reported for the United States and the fifth reported for Florida. It is one of only three sites in the United States with extant populations. Moyroud (pers. comm., December 1997) reports the continuing presence of the Palm Beach County population. I visited the Bay County population on 20 June 1998 and found a single plant with new fronds.

The Franklin County site is within an area regularly botanized by competent botanists. Hence, it is much more likely that the plant is less than three years old than that it has been previously overlooked. I assume that it first appeared following ditch and road improvements made within the past three years.

Precisely how the plant arrived at this location is conjectural. However, hurricane seasons during the mid 1990s have been very active. Four named storms passed through the Florida panhandle during the 1995 season (Allison, Erin, Jerry, and Opal) and another in the 1996 season (Josephine). Allison passed along the Franklin County coast and both Erin and Josephine passed just south of it. It is possible that spores were brought in by one or more of these storms.

This species' limited extent at the site, coupled with its precarious position along a roadside that is often abused by off-road vehicle traffic makes this station extremely fragile and subject to obliteration. Hence, only a single mature frond was collected (*Nelson 502,* FSU). Further collection at or disturbance to the site is not warranted.—Gil Nelson, 4800 Heathe Dr., Tallahassee, FL 32308.

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REVIEW

Common Fossil Plants of Western North America, second edition, by William D. Tidwell. 1998. Smithsonian Institution Press, 470 L'Enfant Plaza, Suite 7100, Washington, DC 20560. xiii, 299 pp. Hardcover (ISBN 1-56098-783-9) $49.95; paperback (ISBN 1-56098-758-8) $24.95.

Most fossil guides seem to focus on the gaudy shells and bones of various animals, and relatively few explore the complexity and beauty of fossilized plants. The first edition of Dr. Tidwell's impressive book was published in 1975 and has long served as a standard reference for fossil hunters in North America. The new edition, which is about 100 pages longer, is a worthy successor that should satisfy the book's many fans.

At first glance, it may not be intuitively apparent why students of pteridology need to have this reference on their bookshelves. However, perusal of the book reveals that it is not merely a collection of pretty plant pictures. Instead, it is a relatively self-contained introduction to major groups of plants that summarizes relationships and morphological/life cycle differences between extant and extinct lineages, and places these groups into a historical context through a table that associates the distributions of various fossil genera with different strata at 95 fossiliferous sites in the western United States and Canada. The book also discusses preservation types and how to collect, curate, and study fossils.

The main portion of the text consists of descriptions and illustrations (an expanded set of beautiful line drawings and both color and black and white photographs) of fossils in various plant groups. A section describing the anatomy of various fossil woods extends the utility of the work beyond the "arm's length" approach to determinations. A well-illustrated glossary and a pictorial summary "outline key" work well to assist users in identifying unknowns.

Pteridophyte groups in the volume are supplemented with a chapter on "fernlike foliage" as well as a brief synopsis of "pseudofossils". The coverage is not just of the more typically seen Carboniferous lycopod and sphenophyte fossils, but also includes short treatments of several modern genera, such as *Dryopteris* and *Onoclea*.

Overall, this volume is attractively packaged, easy to use, and reasonably priced. Its appeal should extend beyond the primary user group of "rock hounds" and paleontologists to those who appreciate the neontological flora and are curious about visible evidence of its ancestry.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

INFORMATION FOR AUTHORS

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American Fern Journal 88(4):145–149 (1998)

Taxonomy and Distribution of *Adiantum trapeziforme* and *A. pentadactylon*

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ABSTRACT.—Data concerning the taxonomy and geographic distribution of *Adiantum trapeziforme* L. and *A. pentadactylon* Langsd. & Fisch. are presented. Both species have been confused in the past and the distinctness between them is discussed.

During the preparation of a taxonomic revision of *Adiantum* (Pteridaceae) in Brazil, the senior author noticed some problems involving the delimitation and distribution of *A. trapeziforme* and *A. pentadactylon*. The former species was described by Linnaeus (1753) and lectotypified by Lellinger (1977) based on an illustration by Sloane (1707: t. 59). Sloane's illustration does not clearly show the diagnostic characters for this species and it might be confused with *A. pentadactylon*, a related taxon. *Adiantum pentadactylon* was described by Langsdorff and Fischer (1810) and its type material (probably an isotype) is at G (photos BM, SP). This material resembles *A. trapeziforme* but represents a different species.

Over the years, several authors have treated these species in different ways, sometimes erroneously. Hooker (1851) mentioned that both species belonged to the *A. trapeziforme* group, which is characterized by: "Fronds tripinnate or decompound; sori almost invariably short, equal or nearly so, rarely continuous or elongated, as in *A. speciosum* and *fumarioides*." He did not, however, clarify the differences between these species. Fée (1869) cited *Glaziou 2318* (B, P) as *A. trapeziforme* f. ß *A. pentadactylon*. Glaziou's material was collected in Rio de Janeiro and is a typical example of *A. pentadactylon*.

According to Baker (1870) in Flora Brasiliensis, *A. pentadactylon* is a synonym of *A. trapeziforme*. Nevertheless, the material cited by Baker, located at BM (*Glaziou 438*) and BR and P (*Glaziou 1754*), are clearly *A. pentadactylon*, and were collected in Rio de Janeiro.

Christ (1900) described *A. trapeziforme* var. *incisum* Christ based on specimens (*Ule 51, 195*) from the State of Santa Catarina, Brazil. The variety was said to differ in its less glaucous pinnules, thinner tissue, segments lobed more than half way to the base, and numerous lobes (6–7 on each side of the segment) usually bifurcate and obtuse. The syntype at P (*Ule 195*), however, is *A. pentadactylon*.

Adiantum pentadactylon was considered a variety of *A. trapeziforme* by Rosenstock (1907). Sehnem (1972) considered Rosenstock's concept wrong and

adopted correctly the name *A. pentadactylon* for plants from the Santa Catarina State. Sehnem presented a clear description and geographic distribution of this species.

Hoshizaki (1970), in a paper about the genus *Adiantum* in cultivation, considered these two taxa as different species and presented some important distinguishing morphological characteristics. According to this author, both species are cultivated largely in the United States. She also mentioned that *A. trapeziforme* is from tropical America and *A. pentadactylon* is from Brazil.

The taxonomic confusion of *A. trapeziforme* vs. *A. pentadactylon* has resulted in some erroneous citations of the former in northeastern Brazil. Pontual (1971) listed *A. trapeziforme* in Alagoas State at northeastern Brazil, but this was based on a misidentification and the species does not occur in this region. The material cited by this author (*Pontual 66/282*) was collected in Santo Antônio farm, at shadow of trees, inside forest and is *A. pentadactylon,* which is rare in remains of the Atlantic Coastal Forest, locally called "Brejos de Altitude."

Adiantum trapeziforme and *A. pentadactylon* were attributed to Pernambuco State (northeastern Brazil) by Barros et al. (1988) who did not cite voucher specimens. We found only cultivated plants of the latter species: *Vieira* s.n. (UFP), *Pickel 628* (IPA, SP), and *Pickel 2119* (IPA). In the State of Pernambuco, the remains of Atlantic Coastal Forest are also present and the occurrence of *A. pentadactylon* is probable.

After examining the literature, types, and specimens from different countries of both species, we conclude that these taxa can be distinguished by the characters presented in the following key:

1. Lamina pinnate; pinnae articulate; petiole color stopping abruptly at segment base; pinnae abaxialy not glaucous; sterile margins shortly crenate . . . . . *A. trapeziforme*
1. Lamina pedate; pinnae not articulate; petiole color passing into segment base; pinnae abaxialy glaucous; sterile margins conspicuously denticulate . . . . . *A. pentadactylon*

Although the lamina architecture can be used to distinguish both species, it is sometimes poorly represented in herbarium material.

Besides morphological characteristics, these species show different geographic distribution patterns in Tropical America. *Adiantum pentadactylon* (Figs. 1B, 2A–E) is an endemic and common species in southeastern Brazil, occurring in the Atlantic Coastal Forest from the State of Alagoas to Rio Grande do Sul. It can be found in the forest usually in wet places from 0–1000 m altitude. Representative specimens are: BRAZIL. **Alagoas**. União dos Palmares, Faz. Santo Antônio, *Pontual 66/282* (IPA). **Minas Gerais**. Juiz de Fora, *Krieger & Roth 1085* (SP). **Rio de Janeiro**. without locality, *Glaziou 438* (BM), *1754* (BR, P), *2318* (BR, P), *7240, 7253* (BR, P). **São Paulo**. São Paulo, Butantã, Rio Pequeno, *Hoehne 2551* (SP). **Paraná**. Guaraqueçaba, Morro do Rio das Pacas, 25°25′S, 48°15′W, *Prado et al. 471* (MBM, NY, UPCB). **Santa Catarina**. Ilha de Santa Catarina, Morro do Antão, *Sehnem 3073* (PEUFR). **Rio Grande do Sul**. Osórios, Lagoa dos Anadros, *Sehnem 5582* (PEUFR).

In contrast, *A. trapeziforme* (Figs. 1A, 2F–H) is a widespread and common

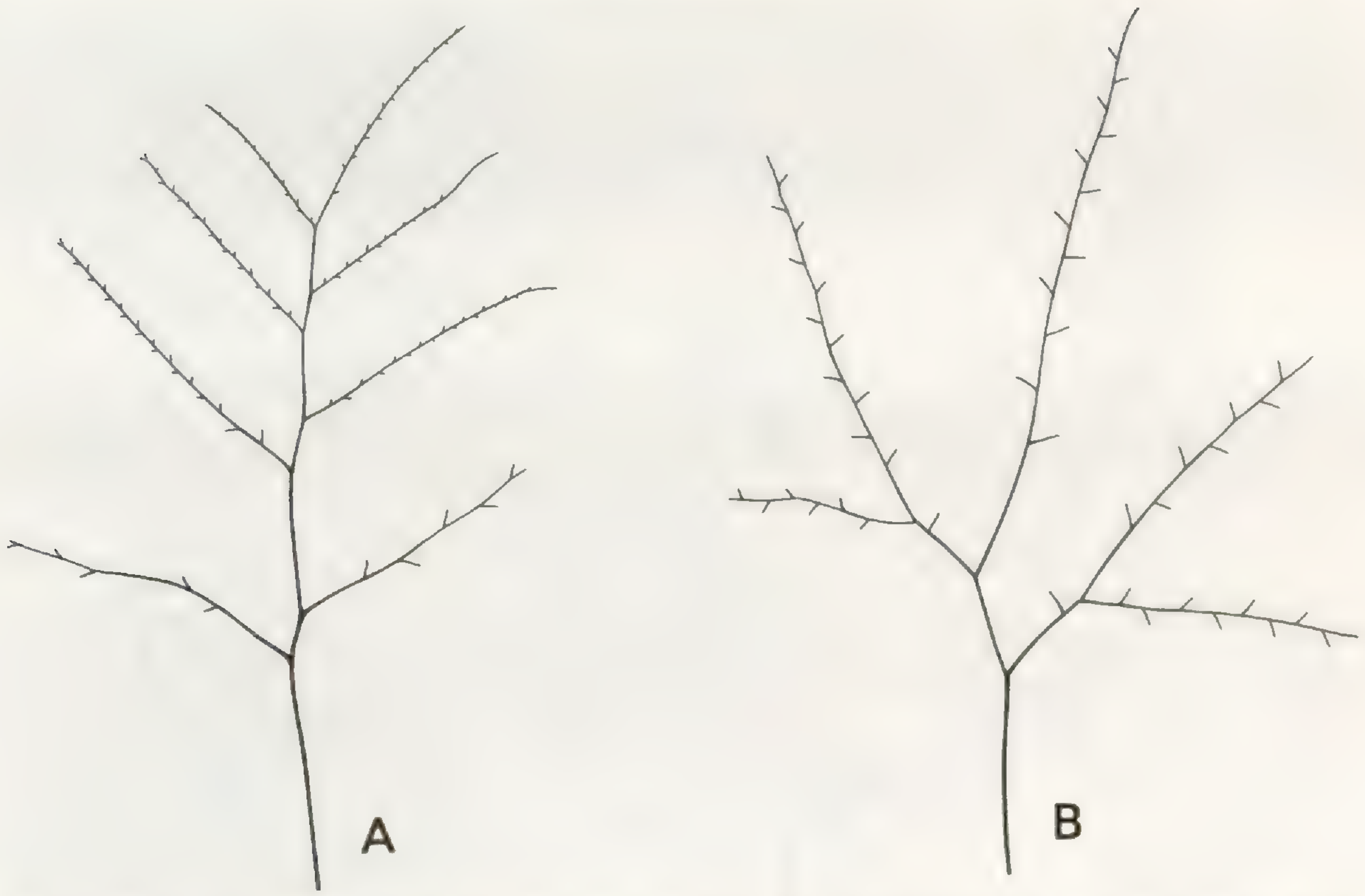

FIG. 1. Schematic representations of lamina divisions in two species of *Adiantum*. A) *A. trapeziforme*. B) *A. pentadactylon*.

species from portions of Mexico through Mesoamerica, Cuba, Jamaica, the Lesser Antilles, Trinidad, and possibly Venezuela (in literature). It grows in wet forests, secondary forests, from lower elevations to 1000 m altitude (Palacios-Rios and Riba, 1983; Diaz-Barriga and Palacios-Rios, 1992). The presence of *A. trapeziforme* in Peru was mentioned by Hieronymus (1909: 237) based on material collected in "Peruvia in declivibus supra tabalosas inter Moyobamba et vallem fluminis Huallaga (*Stübel 1084*)." However, in the recent pteridoflora of Peru by Tryon and Stolze (1989) this taxon was not cited. Specimens we have examined include: MEXICO. **Michoacán**. Aquila, Coalcomán, *Hinton 16301* (DS, NY). **Querétaro**. Landa de Matamoros, 3 km al E. de Sta. Inés, camino a Tangolo, *Fernández 3784* (ENCB, NY). **Veracruz**. Coatepec, *Palacios-Rios 3530* (SP, XAL); Atoyac, Ranchería Caballo Blanco, *Acevedo & F. Vásquez 422* (IEB, XAL); San Andrés Tuxtla, Sihuapan, *Calzada 917* (ENCB, FCME, MEXU, MO, XAL); Cantón de Córdoba, *Conzatti & González 611* (CAS); Orizaba, between Córdoba and Orizaba, on route 150, *Mickel 739* (ENCB, MICH).

In South America there is another related species *A. mathewsianum* Hook. (Fig. 2I) that can be confused with *A. trapeziforme* because of its 3–4-pinnate lamina, articulate, oblong to trapeziform segments, and glabrous petiole and rachis. However, it differs in the firm, rigid, chartaceous texture of its lamina, serrate sterile segment margins, pinnules bases overlaying the rachis, and conspicuously lunulate sori. It occurs from Peru to Paraguay and Brazil (Mato Grosso State).

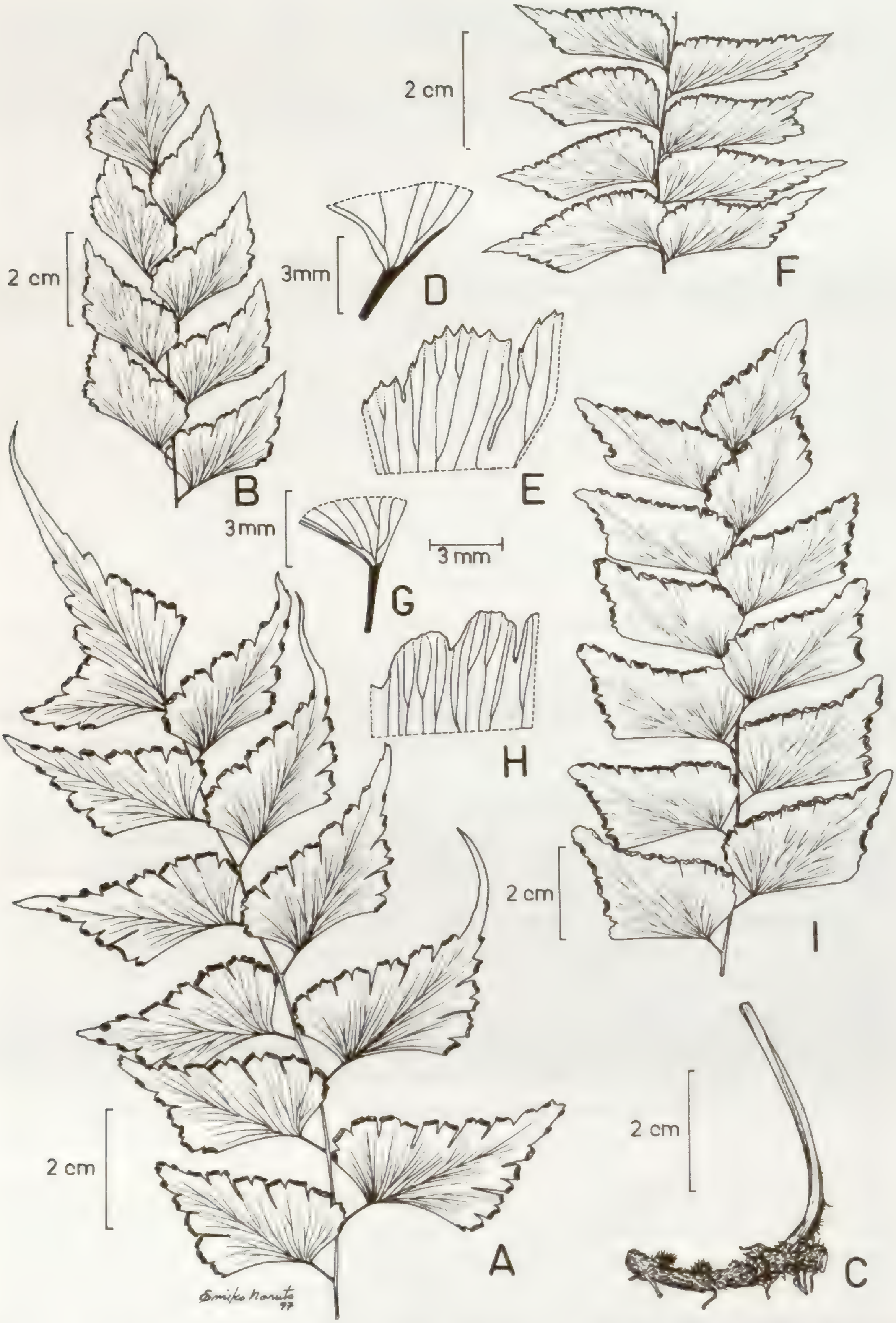

FIG. 2. Comparative morphology of three species of *Adiantum*. A–E) *A. pentadactylon*. A–B) parts of a frond; C) rhizome and petiole base; D) detail of the continuous pinnule; E) margin of the sterile pinnule. F–H) *A. trapeziforme*. F) part of a frond; G) detail of the articulate pinnule; H) margin of the sterile pinnule. I) *A. mathewsianum*, part of a frond.

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American Fern Journal 88(4):150–154 (1998)

Cibotium ×*heleniae* Hyb. Nov. (*C. chamissoi* × *C. menziesii*; Cyatheaceae): A Naturally Occurring Hybrid from Oahu, Hawaii

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ABSTRACT.—The hybrid between Cibotium chamissoi and C. menziesii is described from the Hawaiian island of Oahu. Distinctions between the hybrid and its parents are detailed. The potential existence of other hybrids among the four Hawaiian Cibotium species is discussed.

Intensive investigations of Hawaiian pteridophytes over the past decade have revealed many interspecific hybrids, both published (Ranker, 1992; Wagner, 1993; Wagner et al., 1995; Palmer, 1997) and unpublished. These are recognized usually by their coexistence with the parental species, morphological intermediacy, mostly sporadic occurrence, and more or less abortive spores. Here, I describe a recently discovered natural hybrid between two of the more common Hawaiian species of tree ferns of the genus *Cibotium*.

The genus *Cibotium* includes about ten species ranging from Assam through southeastern Asia to Borneo and the Ryukyus, as well as other Pacific Islands, Mexico, and Central America (Kramer, 1990), with four species recognized in Hawaii (Palmer, 1994). The present paper describes a distinctive hybrid between *Cibotium chamissoi* Kaulf.and *C. menziesii* Hook., presently known only from the island of Oahu, but probably more widespread on other Hawaiian islands.

Cibotium ×heleniae D.D. Palmer, hyb. nov.—TYPE: Hawaii, Oahu, Waialae Nui Ridge, on side of trail, in close association with both putative parents, 520 meters. 26 Jan 1992, *Palmer 812* (BISH—3 sheets).

Planta inter *Cibotium chamissoi* et *C. menziesii,* a *C. chamissoi* palea integer stipae coopertus cum pili lanatus et rectus, et indumentum abaxialis constans pili duo, punctatus et arachnoideus.

Caudex erect ca 16 cm wide, up to 1.5 m long. Blade ca 1.5 m long. Stipe up to 1 m long, stramineous, smooth with occasional dark tubercles, covered entirely with fine tangled reddish-brown hair; hairs wooly, straight or both, wooly hairs tend to be in mats at base and straight hairs more prominent on the distal stipe, occasional short, thick, dark waxy hairs are sometimes present. Lamina bipinnate-pinnatifid to tripinnate, coriaceous, ovate-deltate, ca 150 cm wide at mid-frond, pinnae slightly reduced at base, dark, lustrous, green adaxial surface, dull light-green abaxial surface. Rachis mostly glabrous, stramineous, sometimes hairy, though less so than stipe. Segments of middle portion of middle pinna 12–14 × 5.5–8 mm. Abaxial surface of segments with

TABLE 1. Comparison of *Cibotium chamissoi, C. ×heleniae,* and *C. menziesii.*

Character	*C. chamissoi*	*C. ×heleniae*	*C. menziesii*
Hairs on stipe			
Distribution	base only	entire stipe	entire stipe
Color	golden to mustard	reddish-brown	reddish-brown to dark brown
Character	woolly	woolly, straight, and mixed	straight
Hair cells	flattened	flattened, tubular and mixed	tubular
Dark, waxy, short, thick hairs	absent	scarce	uncommon to common
Segments			
Width	4–5 mm	5.5–8 mm	5–9.5 mm
Adaxial hairs (visible with 20× lens)	arachnoid	arachnoid, punctate and short arachnoid hairs on punctae	punctate
Sinuses			
Depth to costa	7/8+	2/3–7/8	1/3–2/3
Angle	narrow	intermediate	wide

numerous brown-punctate stellate hairs visible with a 20X lens, scattered short arachnoid hair arising from these punctate hairs, and scattered long arachnoid hairs, similar hairs on the costules. Veins generally unforked with once-forked veins more common at distal portion of the frond. Sori 5–7, lining deeper parts of sinuses, containing many club-shaped paraphyses. Spores abortive or not.

PARATYPES.—HAWAII. **Oahu.** Poamoho Trail, on side of trail at ca 1980 feet in association with both parents, *Palmer et al. 1186* (BISH); Aiea Heights-ridge to the summit at ca 500–550 meters, on trail side in association with both species, *Palmer 842* (BISH); Mt. Kaala summit bog at ca. 3995 feet, *Palmer 732* (BISH); Waihole Trail at the base of the Pali at ca 800 feet in close association with both putative parents, *Palmer & Obata 807* (BISH); Mt. Kaala, summit bog at ca 3995 feet, *Palmer et al. 735* (BISH).

Plants of *C. ×heleniae* are generally intermediate between *Cibotium chamissoi* and *C. menziesii* (Table 1). *Cibotium chamissoi* has matted, woolly hairs only at the base of the stipe, whereas *C. menziesii* has stiff straight, unmatted hairs over the entire stipe and on the rachis. The abaxial surfaces of the segments of *C. chamissoi,* for the most part, have only long arachnoid hairs (Fig. 1), and those of *C. menziesii* have only brown, punctate, stellate hairs (Fig. 2). The hybrid is recognized by stipes entirely covered with matted reddish-brown hair composed of both tubular and flattened cells, by intermediacy of pinna size and shape, by the presence of brown punctate hairs (typical of *Cibotium menziesii*), arachnoid hairs (typical of *C. chamissoi*), and short arachnoid hairs arising from the punctate hairs (an intermediate form) on the abaxial surface of the segments. The hair types on the abaxial surface of the segments and the stipe pubescence are the most valuable diagnostic characters; intermediacy in pinna and sinus size and shape are less helpful.

Spores of *C. ×heleniae* vary in morphology, suggesting a partial fertility barrier. Spores from plants collected on the windward side of the island appear abortive; plants on leeward ridges produce spores with a completely formed

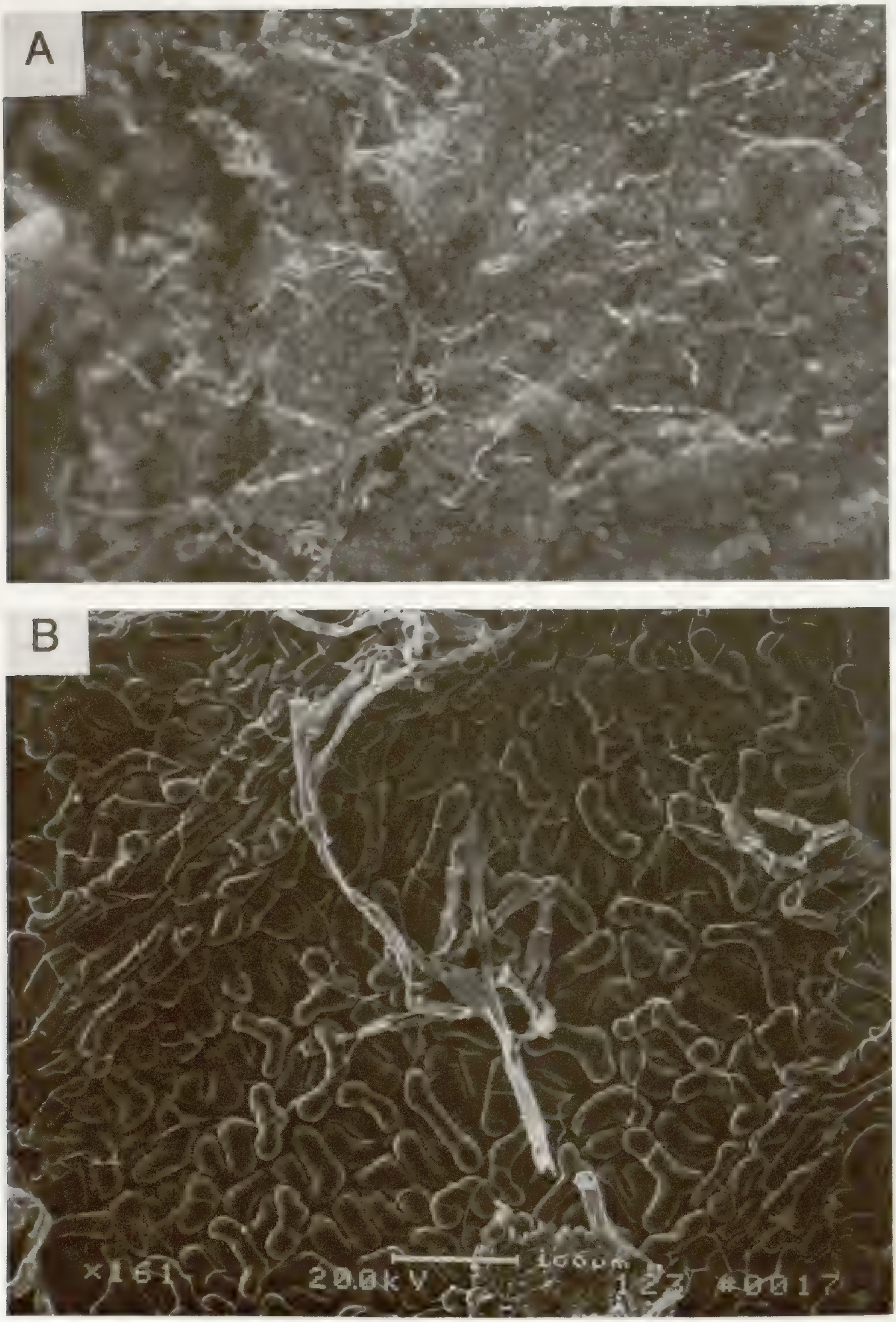

FIG. 1. *Cibotium chamissoi*. A) Arachnoid hairs on the abaxial surface of segments. B) Scanning electron micrograph of arachnoid hairs arising from central punctae (*Degener 23779*, NY).

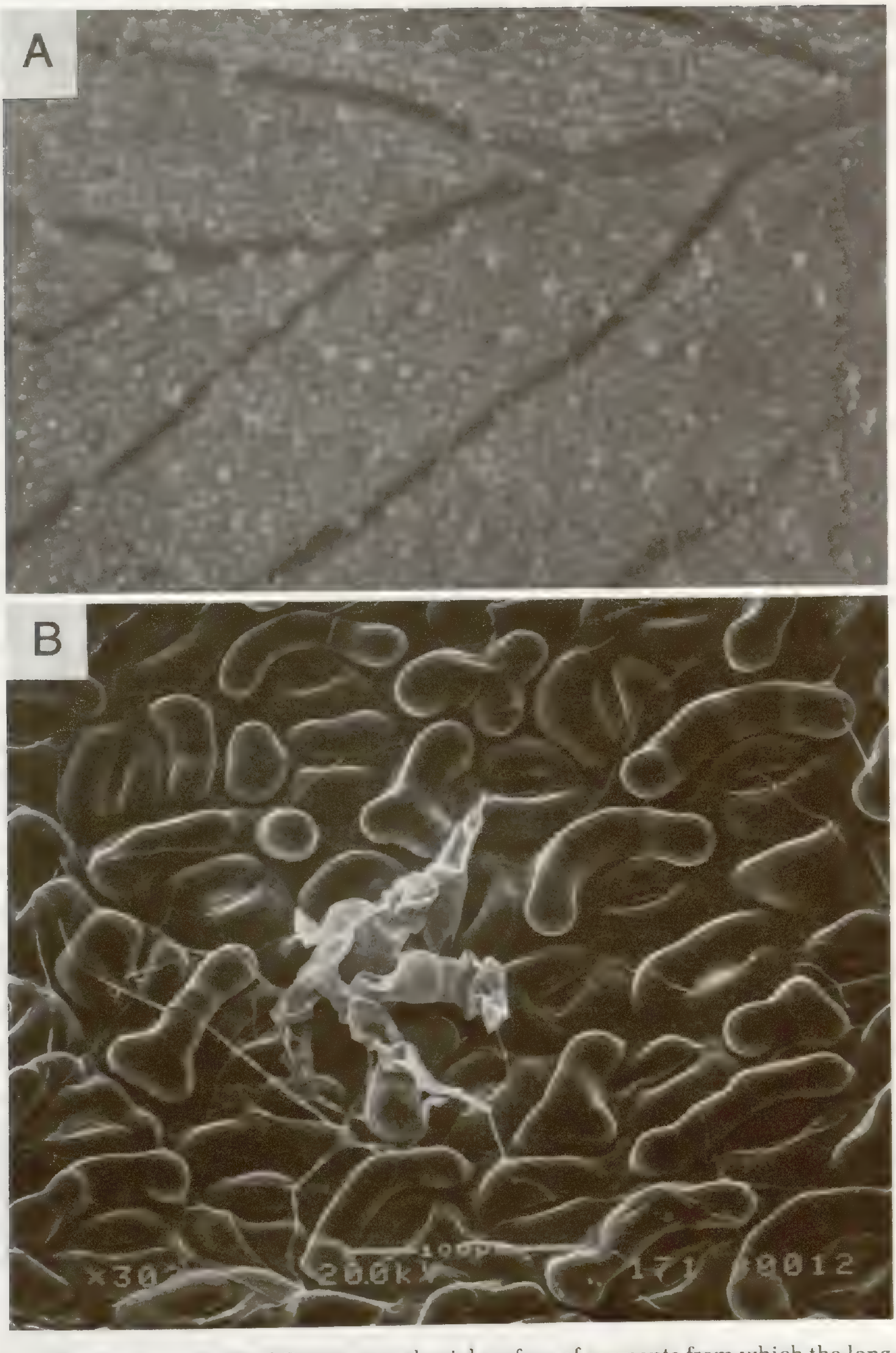

FIG. 2. *Cibotium menziesii*. A) Punctae on abaxial surface of segments from which the long arachnoid hairs seen in *C. chamissoi* arise. B) Scanning electron micrograph of a puncta. (*Little 31142*, NY).

exospore, but transparent with no evidence of cytoplasm; spores collected on Aiea Ridge appear normal and germinate to form gametophytes. The reason for this variability is not known.

Cibotium chamissoi and *C. menziesii* are both common in the lower montane forests and frequently grow together. Hybrids between them are known from several sites on Oahu where both parent species are present in numbers: ca 500 m on leeward ridges of the Koolau Mountains, at the base of the cliffs (pali) on the windward side of the Koolau Mountains, and in the summit bog of Mt. Kaala at ca 1210 m. Although single plants may be found, more often there are several within a 50 m radius, and on Aiea Ridge more than 25 were found.

Other hybrids between the species of Hawaiian *Cibotium* are likely to be found. The hybrids with *Cibotium menziesii* will be most easily detected because of their characteristic stiff straight hairs covering the stipe and rachis and the punctate hairs on the abaxial surface of the segments. Hybrids among the other species will be more subtle and difficult to recognize because they all have woolly hair limited to the base of the stipes, as well as arachnoid hairs on the abaxial surface of the segments. Hybrids between *C. chamissoi* and *C. glaucum* will be most difficult to recognize because of the shared characters of narrow segments and deep narrow angled sinuses.

Just prior to submitting this paper, one plant of the probable hybrid between *Cibotium menziesii* and *C. glaucum* was discovered in the bog of Kohala Mountain on Hawaii Island. It was found in the presence of a large population of both putative parents. *Cibotium chamissoi* does not occur within a few kilometers of the site. This was the only plant of this hybrid recognized by me after extensive surveys in many areas on all the major islands. The spores appear viable and normal in appearance. Efforts are underway to study this hybrid as well.

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American Fern Journal 88(4):155–157 (1998)

A New Species of *Hecistopteris* from Guyana, South America

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ABSTRACT.—First collected in Surinam more than 150 years ago, *Hecistopteris pumila* is known only from tropical America, from a few collections in herbaria, which may be due to its inconspicuous size. A second species, *H. pinnatifida*, was recently described from Ecuador. *Hecistopteris kaieteurensis*, the third species for this genus, is described from Guyana.

For 150 years, *Hecistopteris* was a monotypic genus consisting of the sole species *H. pumila* (Spreng.) J. Sm. known only from tropical America. The genus differs from others in the Vittariaceae by having small (up to ca. 6 cm long), simple, subsessile fronds that lack a pronounced midrib and are apically or laterally lobed or cleft. The plants usually reproduce vegetatively by root proliferations, as illustrated by Kramer (1990, p. 275), on mossy tree trunks. A second species in the genus, *H. pinnatifida* R.C. Moran & B. Øllgaard, was recently described from Ecuador (Moran and Øllgaard, 1995, p.180).

We have found a third species, here named *Hecistopteris kaieteurensis*, while surveying the trees of Kaieteur National Park in Guyana. The main feature of the park is a waterfall with a straight drop of 741 ft. The area at the top of the falls is forested with occasional open "guiana type" savannas that are characteristic of the Roraima formation. The forests are generally along the creeks and rivers and have a canopy ranging from 20–60 meters in height. A small patch of *Hecistopteris kaieteurensis* was found growing in moss on a trunk of a tree approximately 1.5 m above the base. Other trees were not examined at the time to see the extent of the population. According to Jefferson

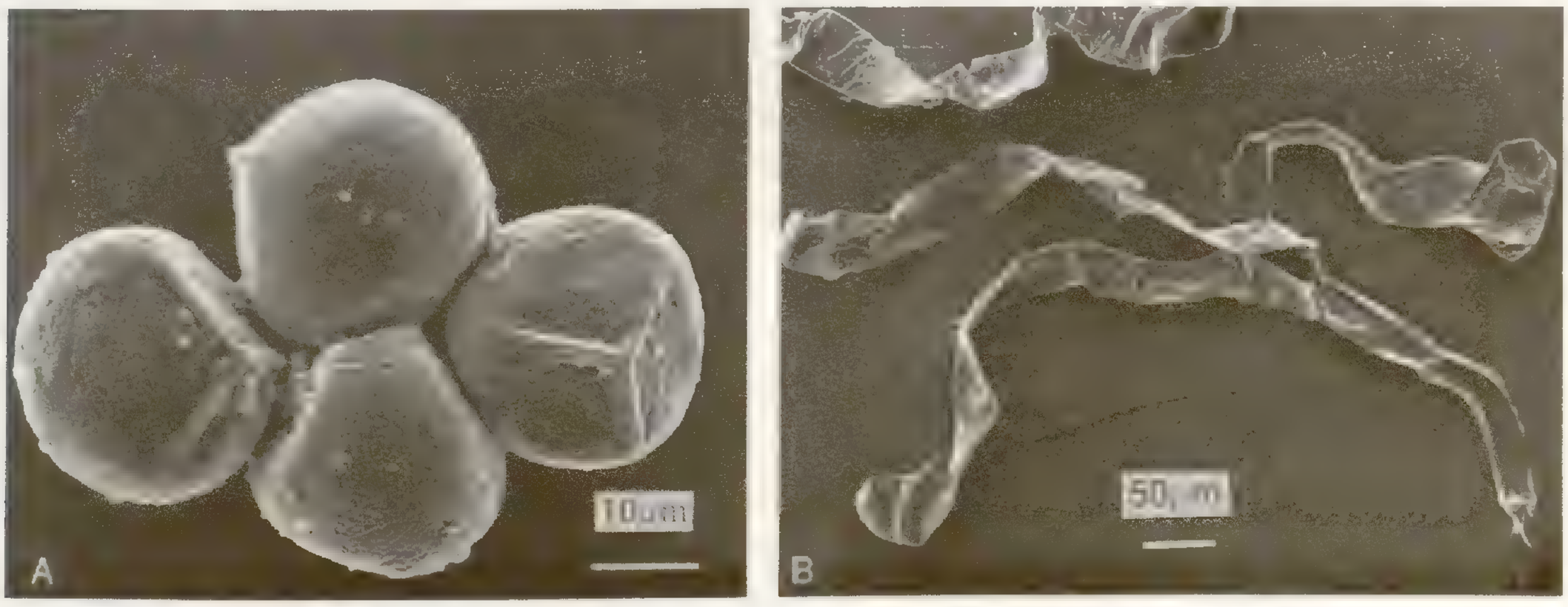

FIG. 1. *Hecistopteris kaieteurensis*. a) SEM photo of trilete spore (x 1.25 k); b) SEM photo micrograph of soral paraphysis showing irregular dichotomization (×245)

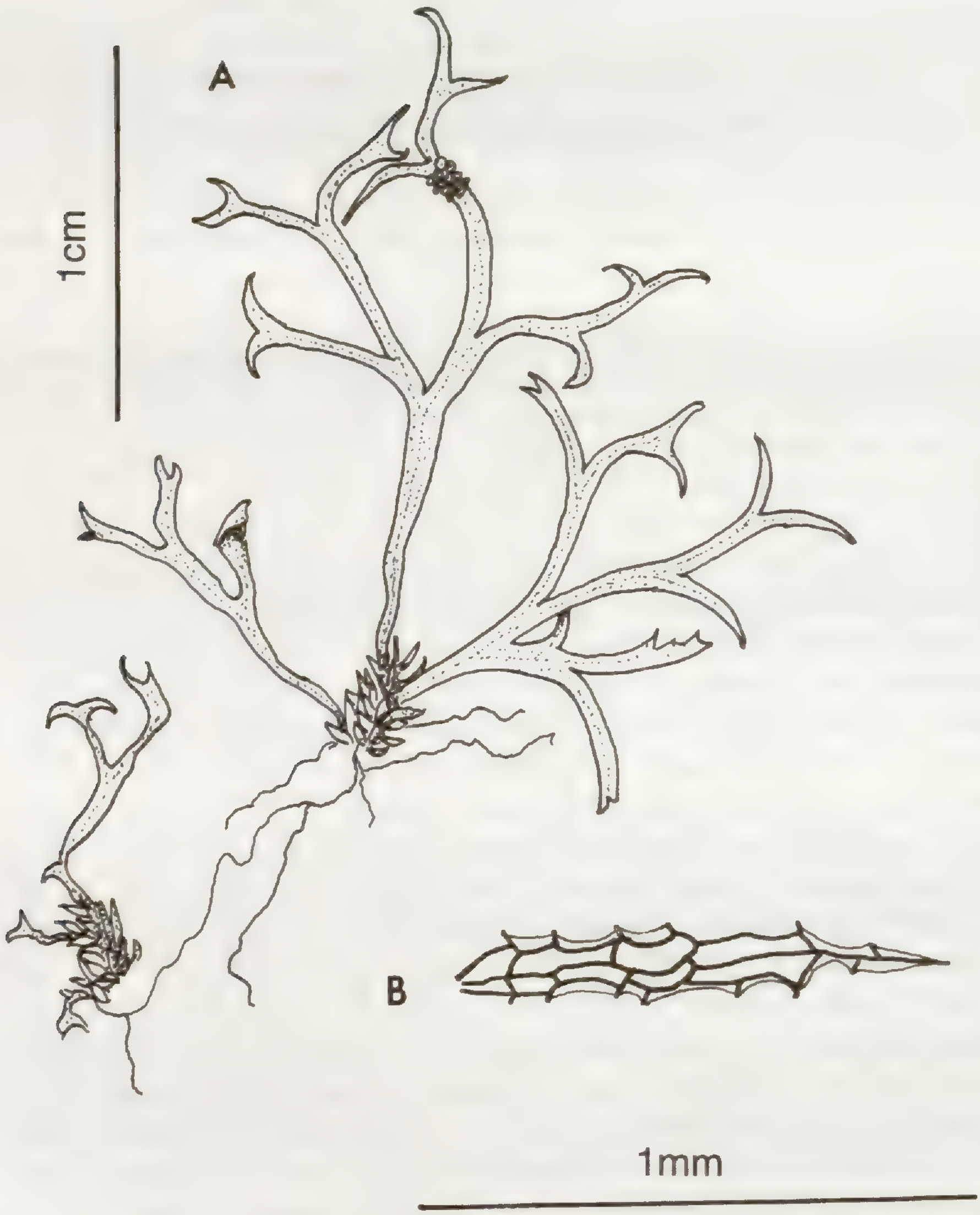

FIG. 2. *Hecistopteris kaieteurensis*. a) habit; b) rhizome scale.

Prado (pers. comm.) this species has been collected near Manaus, Brazil. The habitat is a typical Wallaba (*Eperua* spp.) forest growing on white sand near the headwaters of Korume Creek, Guyana.

Hecistopteris kaieteurensis Kelloff & G. McKee, sp. nov.—TYPE: Guyana, Potaro-Siparuni, Kaieteur National Park, Plot Study #1, NE from end of airstrip at headwaters of Korume Creek. 05°10′N, 59°29′W, 300–350 m elev., 11–19 March 1997, *C. L. Kelloff & G. McKee 1233* (holotype, BRG; isotype, US).

Haec species a *Hecistopteride pumila* (Spreng.) J. Smith rhizomate 1–3 mm longo; squamis 1–2 mm longis rufobrunneis, ad basin 2(4) cellulis latis, adap-

icem 2 cellulis latis; frondibus 0.8–2 cm longis multifurcatis, segmentis 0.5–1 mm latis ad apicem furcatis recurvatisque differt.

Plants epiphytic; rhizomes 1–3 mm long, erect, scaly; scales 1–2 mm long, clathrate, 2(4) cells wide at the base tapering to 2 cells at the apex, reddish-brown; roots elongate, proliferous, bearing copious yellowish root hairs; fronds 0.8–2 cm long, sessile, tapered at base, without a prominent costa, glabrous, several times furcate, the segments 0.5–1 mm wide, furcate with the arms recurved (Fig. 2); veins inconspicuous, forked, free; sori elongate, superficial on the vein in the distal portion of the segments, exindusiate; sporangia short 4-stalked; paraphyses irregularly dichotomizing, or sometimes uniserate, often with an enlargement on the tip of the terminal cells, yellow; spores trilete, yellow (Fig. 1).

Hecistopteris kaieteurensis resembles *H. pumila* in size and habit but can be distinguished from both it and *H. pinnatifida* by its multi-furcate, recurved frond.

Acknowledgments

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Status of *Schizaea pusilla* in New York, With Notes on Some Early Collections

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ABSTRACT.—The occurrence of *Schizaea pusilla* in New York is restricted to eastern Long Island (Suffolk County), where populations occur in moist to wet humus-rich depressions within a dynamic system of shifting dunelands. An historical account of the earliest collections and currently known populations is presented.

Schizaea pusilla Pursh (Schizaeaceae), curly-grass fern, was first discovered in 1805 at Quaker Bridge, Burlington County, New Jersey, by C. W. Eddy, J. Leconte, F. T. Pursh, and C. Whitlow (Redfield, 1876). An intriguing account of the initial discovery and earliest collections has been summarized by Montgomery and Fairbrothers (1992).

Bachelot de la Pylaie (1826), a French botanist, reported *Schizaea* from Newfoundland, but subsequent botanists discounted the report. In 1875, John Redfield reported: "Our little *Schizaea* is known only in the cedar swamps of New Jersey . . . although it is said to have been collected in Newfoundland many years ago." Later, Elizabeth Britton reported: "Dr. Gray . . . wrote to me [in 1879] that he had seen long ago, the specimen collected by De la Pylaie in Newfoundland, in the Herbarium of the Jardin des Plantes at Paris, but that everyone had supposed that the locality cited was incorrect, and that the specimens must have come from New Jersey" (Britton, 1896a).

In July 1879, Elizabeth Knight [Britton] collected *S. pusilla* from near the shores of Grand Lake, Nova Scotia (Eaton, 1879a, 1879b; Gray, 1880). In September 1895, she collected it in Newfoundland (Britton, 1896b) and noted: "The rediscovery of so interesting a plant after so long an interval of time has elapsed, shows that the plants are there but fail to be recognized. It may now be looked for with renewed hope of finding it along the coast from Maine to Long Island [New York]."

By 1910, numerous populations of *S. pusilla* had been located in Newfoundland. Fernald (1911) reported: "Among the tufts of *Scirpus caespitosus* . . . was the famous little fern of the New Jersey Pine Barrens, *Schizaea pusilla,* already known in Newfoundland from collections of la Pylaie, Waghorne, and Eames & Godfrey and after we had once seen it found [it] wherever we looked for it; also known at remote stations in Nova Scotia, but quite unknown between there and southern New Jersey." Fernald's expedition to Nova Scotia in 1920 revealed additional populations of *S. pusilla* in Digby, Yarmouth, Queens, and

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Halifax Counties, but he reiterated, "Between the pine barrens of New Jersey and Nova Scotia *Schizaea* is quite unknown, although repeatedly sought on Long Island, Nantucket and Cape Cod . . ." (Fernald, 1921).

On 12 September 1927, Roy Latham collected *S. pusilla* from a "moist cranberry bog" near Montauk on the east end of Long Island, Suffolk County, New York. Two years later, on 30 August 1929, he located a second population occurring in "wet sandy ground," also near Montauk. Years later Latham described the locality of the populations in two letters to Henry Bookout: "I used to take the first lane to the ocean beach east of Napeague Harbor, I think it is called Napeague Lane. I left my car on that lane and walked east perhaps a mile. It is a considerable area with wet, sandy bogs full of cranberry plants and small plots of pines in wet sandy ground. It was along the edge of these pine stands that I found two small colonies of the *Schizaea pusilla,* not together. Both colonies were west of the next lane to the ocean, east of Napeague Lane. But what has been done there since my collecting days, I have no knowledge now . . . I took a few specimens of the curly-grass from each colony. I think they went to Albany with all my notes and records . . ." (Latham, 1970a). This locality is south of Montauk Highway (Route 27) approximately 4 miles west of the town of Montauk; the land is now part of Hither Hills State Park. Latham's second letter to Bookout described the locality of another population of *S. pusilla*: "Thank you for the photo of *Schizaea.* I am pleased that you discovered it there. I thought that it was scattered all over that area and especially north and west of Fresh Pond where the *Habenaria cristata* is so common." (Latham, 1970b). The Fresh Pond locality is north of Montauk Highway in Hither Hills State Park, approximately one mile northwest of the other population. The general location of these two populations is also confirmed by a third letter from Latham to Robert Dirig: "I did find the curly grass in two places on Montauk, one patch south of the highway and the other in the far western end, near the bay." (Latham, 1976).

For some unexplained reason, Latham failed to publish a report of the discovery of *S. pusilla* on Long Island. Latham had been a prolific collector of vascular plants, was intimately familiar with botanical literature, and published extensively in scientific journals. Dirig (1994) credited Latham with more than 300 published papers on various natural history disciplines; Lamont (1993) provided a bibliography of Latham's 27 botanical publications. Latham's voucher specimens of *S. pusilla* remained in his personal herbarium until 1969, when his entire collection was donated to the New York State Museum (Richard Mitchell [NYS], pers. comm.).

The initial discovery of *Schizaea* on Long Island went unnoticed by the botanical world for more than 30 years (see Table 1). Small (1935) noted in *Ferns of the Vicinity of New York:* "Now that *Lycopodium carolinianum* has been discovered on Long Island, careful search for *Schizaea* should be made wherever that lycopod grows." In *Gray's Manual of Botany* (Fernald, 1950), curly-grass fern is listed as occurring in Newfoundland, Nova Scotia, and the pine barrens of New Jersey. Likewise, in *The New Britton and Brown Illustrated Flora,* Morton (1952) reported curly-grass fern as rare and local in New-

TABLE 1. Summary of significant collections of *Schizaea pusilla,* including pertinent reports from publications.

1805:	Curly-grass fern is discovered near Quaker Bridge, Burlington County, New Jersey, by C. W. Eddy, J. LeConte, F. T. Pursh, and C. Whitlow.
1814:	F. Pursh describes *Schizaea pusilla* in his *Flora Americae Septentrionalis.*
1826:	A. Bachelot de la Pylaie, a French botanist, reports *Schizaea* from Newfoundland; but subsequent botanists supposed that the locality cited was incorrect, and that the specimens must have come from New Jersey.
1847:	A. Wood reports *S. pusilla* from Western, New York; but in 1867 he retracts the report.
1879:	E. Knight [Britton] collects *S. pusilla* from near the shores of Grand Lake, Nova Scotia.
1895:	E. Britton rediscovers *S. pusilla* in Newfoundland, and concludes: "It may now be looked for with renewed hope of finding it along the coast from Maine to Long Island [New York]."
1921:	M. L. Fernald notes: "Between the pine barrens of New Jersey and Nova Scotia *Schizaea* is quite unknown, although repeatedly sought on Long Island, Nantucket and Cape Cod . . ."
1927:	R. Latham collects *S. pusilla* on eastern Long Island, New York, but fails to publish a report of the discovery.
1935:	J. K. Small notes: "Now that *Lycopodium carolinianum* has been discovered on Long Island, careful search for *Schizaea* should be made wherever that lycopod grows."
1945:	H. Brown reports an historical collection of *S. pusilla* from Ontario, Canada; but there may have been confusion about the location.
1960:	H. Moldenke publishes the first New York report of *S. pusilla* from eastern Long Island.
1987:	R. Stolze reports *S. pusilla* from Peru; Wagner (1993) suggests that this taxon may be a "closely related species."
1990:	F. Hirst reports *S. pusilla* from Sussex County, Delaware.

foundland, Nova Scotia, Ontario, and New Jersey (the report from Bruce County, Ontario [Brown, 1945], has been discounted by subsequent authors [see Wherry, 1961; Stolze, 1987; Montgomery and Fairbrothers, 1992; Wagner, 1993]).

On 23 July 1960, Harold and Andrew Moldenke located a third population of *S. pusilla* on eastern Long Island and published the first report from New York State (Moldenke, 1960). The population was located in a "small cranberry bog near Montauk, Suffolk County . . . in association with cranberry, three species of sundew (*Drosera intermedia, D. rotundifolia,* and *D. filiformis*), and marsh St. Johnswort (*Triadenum virginicum*)." This new site was located at Napeague State Park, almost 5 miles west of the Latham populations and approximately 3 miles east of Amagansett. Twelve years later, H. Moldenke wrote: "I am so glad that the *Schizaea* station is still there and flourishing. Not having been able to locate it again myself, I was beginning to fear that it had been wiped out and that perhaps we would be blamed for having done so. Actually, now that I have been thinking about it, we took *two* specimens—one went to the herbarium of the British Museum and the other to that of the Texas Research Foundation in Renner (for whom I do a good bit of collecting)—so vouchers are in two institutions" (Moldenke, 1972).

During the 1960s, few botanists visited the new *Schizaea* site at Napeague State Park, and the original Latham populations at nearby Hither Hills State Park still went unreported. Stanley Smith, former Curator of Botany of the New

York State Museum at Albany, visited the Napeague site during the 1960s but did not collect specimens of *S. pusilla* (Mitchell, pers. comm.).

Numerous botanists visited the Napeague site during the 1970s, and collections of *S. pusilla* were deposited in several herbaria (see specimens examined, below). At this time, unsuccessful attempts were made to relocate Roy Latham's two populations at Hither Hills State Park.

Joseph Beitel showed me the *Schizaea* site at Napeague State Park during the late 1970s and Henry Bookout showed me another nearby site some years later. One population occurred in a small cranberry bog north of Montauk Highway. Another population occurred in a long, open moist swale that McKeever (1971) described as "undoubtedly the bed of the old wagon road to Montauk." At least two additional small populations also occurred within the immediate vicinity.

Sometime shortly after 1981, one population at Napeague State Park (just north of the railroad tracks) died out and has never recovered. A drought on Long Island in 1980 accompanied by slight changes in water drainage patterns near the site may account for the loss of this population. Subsequent surveys of the site have revealed that it has been slowly succeeding into a shrubland and is consistently drier than it used to be.

Throughout the 1980s and early 1990s, the "old wagon road" population at Napeague State Park remained relatively stable, with the population size fluctuating between at least 40 to more than 100 individuals.

All of the Long Island populations of *S. pusilla,* including the currently known ones at Napeague State Park and the historical ones at Hither Hills State Park, occur in moist to wet sandy depressions within a dynamic system of shifting dunelands bordering the Atlantic Ocean and Gardiners Bay. The ecology of these plant communities has been described in detail by Johnson (1981, 1985). The low, undulating dunelands are dominated by an unusual growth form of *Pinus rigida,* in which the lower branches surrounding the upright trunk grow out horizontally over the sand like a blanket. Other trees occurring with the pines include *Quercus alba, Q. coccinea, Q. stellata, Q. velutina,* and *Prunus serotina.*

Widely dispersed throughout the pitch pine-oak duneland forest are wet to moist, open depressions. The underlying sandy soil is overlayed with rich humus that is low in mineral nutrients and intensely acidic (Johnson, 1985). During years of above average precipitation, these depressions often become flooded for periods of time ranging from several weeks to several months. *Sphagnum palustre* and *Vaccinium macrocarpon* dominate these depressions, along with a high diversity of herbaceous species including: *Andropogon glomeratus, Calopogon tuberosus, Cladium mariscoides, Drosera filiformis, D. intermedia, D. rotundifolia, Euthamia tenuifolia, Iris prismatica, Juncus canadensis, Lycopodiella alopecuroides, L. inundata, Panicum virgatum, Pogonia ophioglossoides, Polygala cruciata, Rhexia virginica, Rhynchospora alba, R. capitellata, Scirpus pungens, Triadenum virginicum, Viola lanceolata,* and *Xyris torta.*

The current population of *S. pusilla* at Napeague largely occurs along the

slightly elevated, south-facing margins of a long, humus-rich, acidic depression and on the edges of hummocks within the depression. It does not usually grow in dense mats of *Sphagnum,* nor in the dense shade of shrub thickets. Lellinger (1985) stated that *S. pusilla* usually occurs in association with *Chamaecyparis thyoides,* but such is not the case on Long Island.

In 1995, Long Island experienced another severe drought. A local weather station at Montauk recorded precipitation totals for the first 8 months of the year that were 40.2% below normal; no precipitation was recorded for 28 consecutive days from 6 August 1995 to 2 September 1995 (K. Hicks, pers. comm.). The long moist swale at Napeague (old wagon road site) dried up and baked in the summer heat. The immediate effects of the drought on the *Schizaea* population were devastating. During several visits to the site that summer I was able to locate only six plants, and none had produced fertile fronds.

Average amounts of precipitation were recorded on Long Island during late 1995 and the first six months of 1996, but intense searches by several botanists revealed only two individuals of *S. pusilla* at the old wagon road locality in Napeague. It was feared that New York might lose its last known population of curly-grass fern.

Monthly surveys from June to October 1997 revealed a recovery of *S. pusilla* at the Napeague site (Lamont, 1997). Twenty-four individuals were observed at the old wagon road locality and a new sub-population, consisting of three individuals, was discovered 100–125 m east of the main population. Approximately half of the individuals had produced fertile fronds.

Twenty years of field observations suggest that *S. pusilla* can tolerate a wide range of moisture gradients. Apparently, the sporophyte can survive for at least ten weeks submerged under water, whereas the gametophyte can sustain limited periods of drought. It has been previously demonstrated in culture that the gametophyte can survive below freezing temperatures (Atkinson, 1965). Small (1935) noted, "The fern has a wide range of resistance to extremes of temperature—heat in southern New Jersey; cold in Newfoundland." The anatomy of the gametophyte and sporophyte were first described in detail by Britton and Taylor (1901). Spore germination requirements have been described by Guiragossian and Koning (1986).

Because large tracts of appropriate habitat remain available to support populations of *S. pusilla,* it is possible that overlooked populations still occur on eastern Long Island. Conversely, wind-blown sand and shifting dunelands do encroach upon and consume scattered, moist depressions; such may have been the fate of the historical populations of *S. pusilla* at Hither Hills State Park.

SPECIMENS EXAMINED.—NEW YORK. Suffolk Co., Montauk, moist cranberry bog, 12 Sep 1927, *Latham* s.n. (NYS); Montauk, wet sandy ground, 30 Aug 1929, *Latham* s.n. (NYS); Napeague, NW corner of a sandy bog, with *Lycopodium alopecuroides* and cranberry, N of the Montauk Hwy, elev. ca. 10 ft, 23 Aug 1970, *Bookout 162* (CU, NY, photo at BKL); E of Amagansett, Township of East Hampton, small cranberry bog, N of Montauk Hwy, 20 Aug 1973, *Beitel 44* (NY); Napeague, small cranberry bog, hummocks with pitch pine, N of

Montauk Hwy, 23 June 1975, *Johnston 263* (OBPF); 9 Sep 1975, *Johnston 264* (OBPF); E of Amagansett off Hwy 27, on tiny *Sphagnum* patches at edge of small clear water areas in little bogs, 1 Sep 1976, *Mitchell* & *Dean 5606* (NYS); Napeague, Town of East Hampton, N of Montauk Hwy (Rte. 27) on boggy flat, elev. ca. 5 ft., 20 Aug 1981, *Cryan & Dirig 473* (CU).

Acknowledgments

Appreciation is expressed to the curators and staff of BKL, HUH, NY, NYS, and OBPF for kindly making collections available for study. For providing label data on *Schizaea* collections deposited at CU, I thank Robert Dirig, and for searching for Long Island *Schizaea* collections at PH, I thank Ted Gordon and Alfred Schuyler. For assistance in the field, I thank James Ash, Henry Bookout, and Ann Johnson. I also thank John Mickel and James Montgomery for reviewing an earlier draft of the manuscript. I am especially indebted to the late Joseph Beitel for first showing me the *Schizaea* site at Napeague State Park.

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Old World Climbing Fern (*Lygodium microphyllum*), a Dangerous Invasive Weed in Florida

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ABSTRACT.—*Lygodium microphyllum*, a native of the warm and wet regions of the Old World, was first detected to be naturalized in southeastern Florida in 1965. This fern has become an aggressive invader of natural vegetation in many different habitats that are frequently dominated by the weed. Aerial surveys conducted in 1993, 1995, and 1997 detected increasing densities and continued expansion of its distribution in Florida. The fern is expected to continue to increase in Florida and could spread by spores to suitable habitats in Texas and Mexico. No effective method of control for the plant exists.

Lygodium microphyllum (Cav.) R. Br. (Schizaeaceae), or Old World climbing fern, is native to wet tropical and subtropical regions of the Old World. It has become a serious weed in southeastern Florida, where it is increasing in density and range. In the present paper we discuss the ecological problems associated with the fern and its naturalization and spread, probable origin, and potential to spread, and the control attempts to date. The companion paper (Pemberton, 1998) discusses the potential of biological control to reduce populations of this plant and to limit its spread. The fern's native distribution and taxonomic relations, both essential information for a biological control program, are also reported.

THE PROBLEM

Lygodium microphyllum is a "kudzu-like" vine that invades many freshwater and other moist habitats in Florida (Fig. 1). The "stems," which are actually single compound fronds with indeterminate growth, climb high into and over the tops of trees. The fern's ability to grow over trees and shrubs and to run horizontally smothers whole communities of plants (Fig. 2). It is difficult for other plants to grow through the thick fern mats. The dominant fern growth reduces plant diversity. It is common in bald cypress *(Taxodium distichum* (L.) Rich.) stands, but also infests pine flatwoods, wet prairies, saw grass (*Cladium jamaicense* Crantz) marshes, mangrove communities, Everglades tree islands, and disturbed areas. Some Everglades tree islands are so completely blanketed by the fern that it not possible to see the trees and other vegetation beneath it. *Lygodium microphyllum* also threatens rare plants. In Loxahatchee Slough in Palm Beach County, the fern is an "imminent danger" to *Tillandsia utriculata* L. and other rare bromeliads (Craddock Burks, 1996).

FIG. 1. *Lygodium microphyllum* showing its sterile unlobed pinnae with entire margins and fertile pinnae with sporangia bearing teeth.

Infestations of this fern alter the impact of fire, which is a naturally occurring element and a management tool in many Florida communities (Roberts, in press). Thick skirts of old fronds enclose trees and serve as ladders that carry fire into tree canopies. Trees that can withstand ground fires are killed when the fire is brought to the canopy. Fires that usually terminate at the margins of cypress sloughs during the wet season can burn into and through cypress sloughs infested with the fern. Portions of the burning fern can also break free and kite the fire to new areas. The fern appears to be long-lived and persistent. At the Reese Groves property in Palm Beach County, the fern has been present for 20 years. A mat composed of above ground stems and underground root mass has formed that is more than one meter deep.

Lygodium microphyllum can occupy a large proportion of suitable habitats

FIG. 2. Infestation of *Lygodium microphyllum* in a cypress grove at Dickinson State Park in southeastern Florida. Note the fern's horizontal mat of growth smothering the understory vegetation in the foreground and its climbing growth on trees in the background.

in a relatively short time. It was first collected from Jonathan Dickinson State Park in Palm Beach County in 1966 (Beckner, 1968), and by 1993 it was present on 1,233 acres (11%) of the park and the Loxahatchee National Wild and Scenic River (Roberts, in press). In 1995, the plant was present on 17,000 acres (12%) of the Loxahatchee National Wildlife Refuge (Palm Beach County), where it was undetected in 1990 (Sue Jewel, pers. comm).

Lygodium microphyllum is also known to be weedy in Malaysia (Holttum, 1959). It is classified as a principal weed in that country and a weed of unknown importance in Vietnam (Holm et al., 1979).

NATURALIZATION AND SPREAD

The earliest collection of *L. microphyllum* as a naturalized plant in the New World was in Jamaica in 1958 (*Proctor 18421,* NY). The collection was near Castleton Gardens, where an earlier specimen was taken from a cultivated plant in 1926 (*Maxon 10511,* NY, misidentified as *L. flexuosum* (L.) Sw.). The fern was also found to be naturalized in Guyana in 1992 (*Hoffman and Capellaro 776,* NY). It was first collected as a naturalized plant in Florida at two Martin County locations in 1965 and 1966 (Beckner, 1968). In 1973, the fern was collected in Highlands County to the northwest across Lake Okeechobee from Martin and Palm Beach County (*Alverez* s.n.; *Avery 1427,* FLAS). In "The

Ferns of Florida," Lakela and Long (1977) stated that this plant was rare and that a sporulating specimen had been recorded from Martin County. In 1978, *L. microphyllum* was known from 16 localities in Martin County and 23 in Palm Beach County (Nauman and Austin, 1978). At the time, this fern occurred in great abundance for several miles along the Florida Turnpike in Martin and Palm Beach County (*Godfrey 76935,* FLAS, specimen notes). In 1973, the plant was collected in Highlands County (*Alvarez P7718,* FLAS) and in Polk County to the north of Highlands County (*Willson 353,* FLAS) in 1979. In 1981, it was documented to be naturalized in Collier County on the Gulf of Mexico coast in southwestern Florida (*Robinson, Wunderlin, Hansen & Tloenke 207,* USF).

In 1993, the South Florida Water Management District initiated biennial aerial surveys to determine the distribution, density, and spread of four invasive weeds: *Melaleuca quinquenervia* (Cav.) S.T. Blake, *Schinus terebinthifolius* Raddi, *Casuarina* spp., and *L. microphyllum*. Fifty flight lines were established for the entire area south of the north rim of Lake Okeechobee. The lines were spaced at 2.5 mile intervals, in an east/west pattern across the state. The beginning and end point of each line is a precise point of latitude and longitude to permit repeat surveys.

Surveys were conducted in 1993, 1995, and 1997. Observers were trained in detecting the exotic plant survey targets from the air. Each survey team consisted of two observers and a pilot. The fixed-wing aircraft (a 172 Cessna) was equipped with three Global Positioning Systems (GPS) units and two data recorders. The pilot used one GPS unit to navigate along the calculated flight line. The observers—stationed on opposite sides of the plane—recorded the occurrence and density of the target species at eight second intervals. *Lygodium microphyllum* has a characteristic light green color, which enables it to be easily seen from the air.

Figures 4–6 show the occurrence of *L. microphyllum* in 1993, 1995, and 1997. For comparative purposes, we created a pseudo-survey map for 1978 (Fig. 3) by transposing the Nauman and Austin (1978) locality data for Martin and Palm Beach County onto the flight lines used for the surveys. These four figures provide a time sequence of the spread and increase in density of this fern in southern Florida.

In 1978, *L. microphyllum* was limited to eastern third of Martin and Palm Beach Counties (and Highlands County to the north of the survey area). By 1993, the fern expanded into western Martin County, central Palm Beach County, and was detected in Glades County, northwest of Lake Okeechobee. The total estimated infested area in 1993 was 11,213 hectares (27,686 acres). In 1995, *L. microphyllum* was first detected to the south in Broward County, to the west in Hendry County (in the west central south Florida), and in Sarasota and Collier Counties on the west coast. The infested area was estimated to be 11,831 hectares (29,212 acres) in 1995. In 1997, the fern was detected in Lee and Charlotte Counties, and had covered an estimated 15,892 hectares (39,240 acres). Populations detected at four well separated sites in Charlotte County, where none were detected in 1995, are particularly striking.

The known distribution of the fern in Florida is shown in Fig. 7. The map

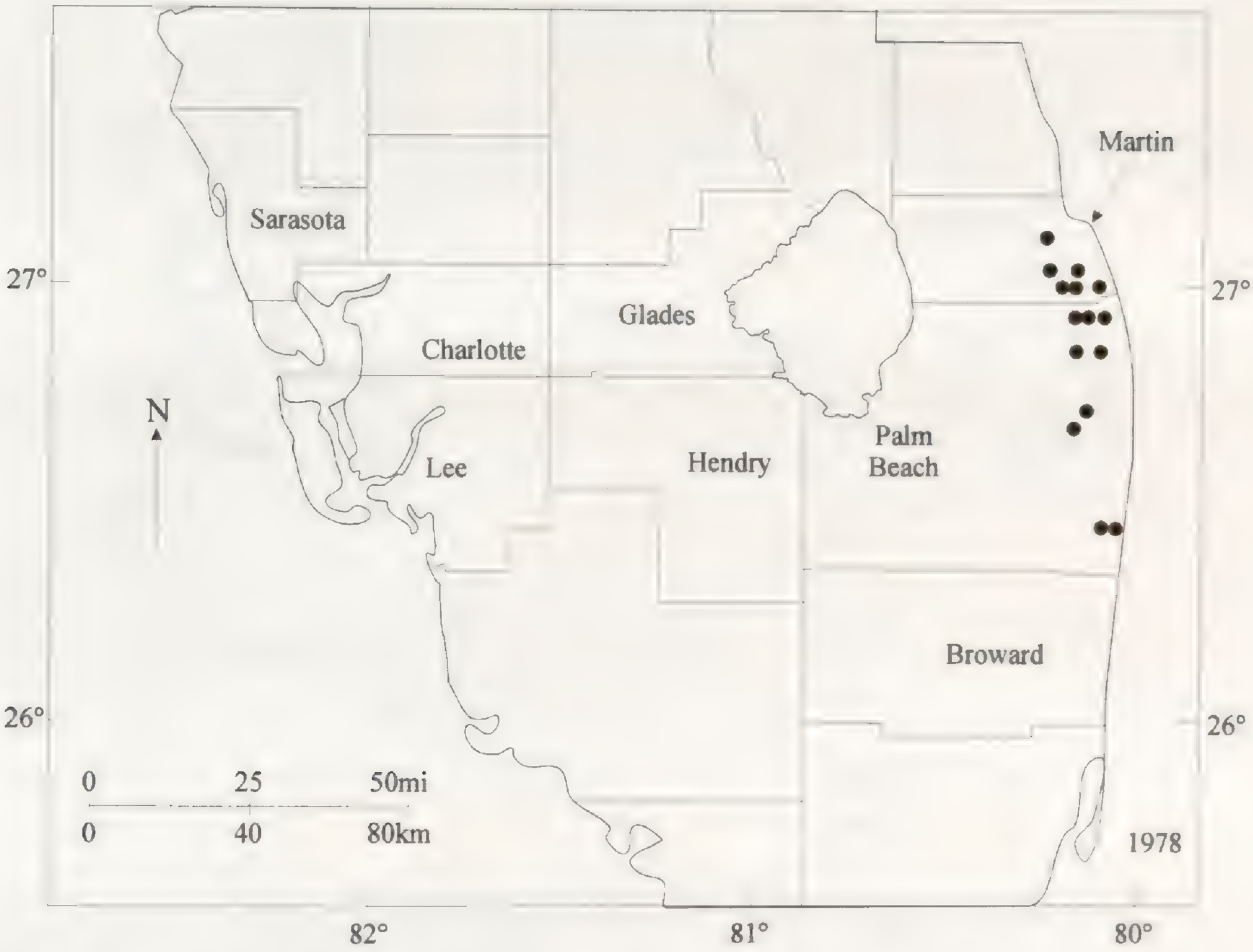

FIG. 3. Distribution of *Lygodium microphyllum* infestations in southern Florida in 1978. Data from Austin and Nauman (1978) transposed onto flight lines flown during aerial surveys during 1993, 1995, and 1997.

indicates sites detected during the aerial surveys and by collections documented by herbarium specimens. The herbarium records not discussed above are: Polk County in 1981 (*Lindsey & Upchurch 457,* USF); Desoto County in 1991 (*Mears* s.n., USF 202986); Lee County in 1992 (*Clark* s.n., USF 204553); Manatee County in 1992 (*Cole WC0087,* USF), and Hardee County in 1996 (*Ferriter* s.n., USF 218483). Recently, in 1997, the fern was detected in Brevard County (M. A. Poole, pers. comm.), about 50 miles (ca. 80 km) north and east of known populations. *Lygodium microphyllum* appears to be rapidly expanding its range and increasing in density.

The apparent pattern of spread from the east coast inland to sites northwest and west, and then west to west coast sites is the pattern expected if the spores were carried by the prevailing winds of spring, summer, and autumn. The prevailing winds in Palm Beach and Martin Counties during the spring and summer are from the southeast and those of the autumn are from east northeast (Henry et al., 1994). Although spore production appears to occur year round, spore production and dispersal patterns are unknown. Preliminary data suggest that there are large numbers of *L. microphyllum* spores in the air. In one Martin County infestation, 724 spores/m^3/hour were captured with a Burkhardt portable personal pollen and spore sampler (Pemberton, unpublished

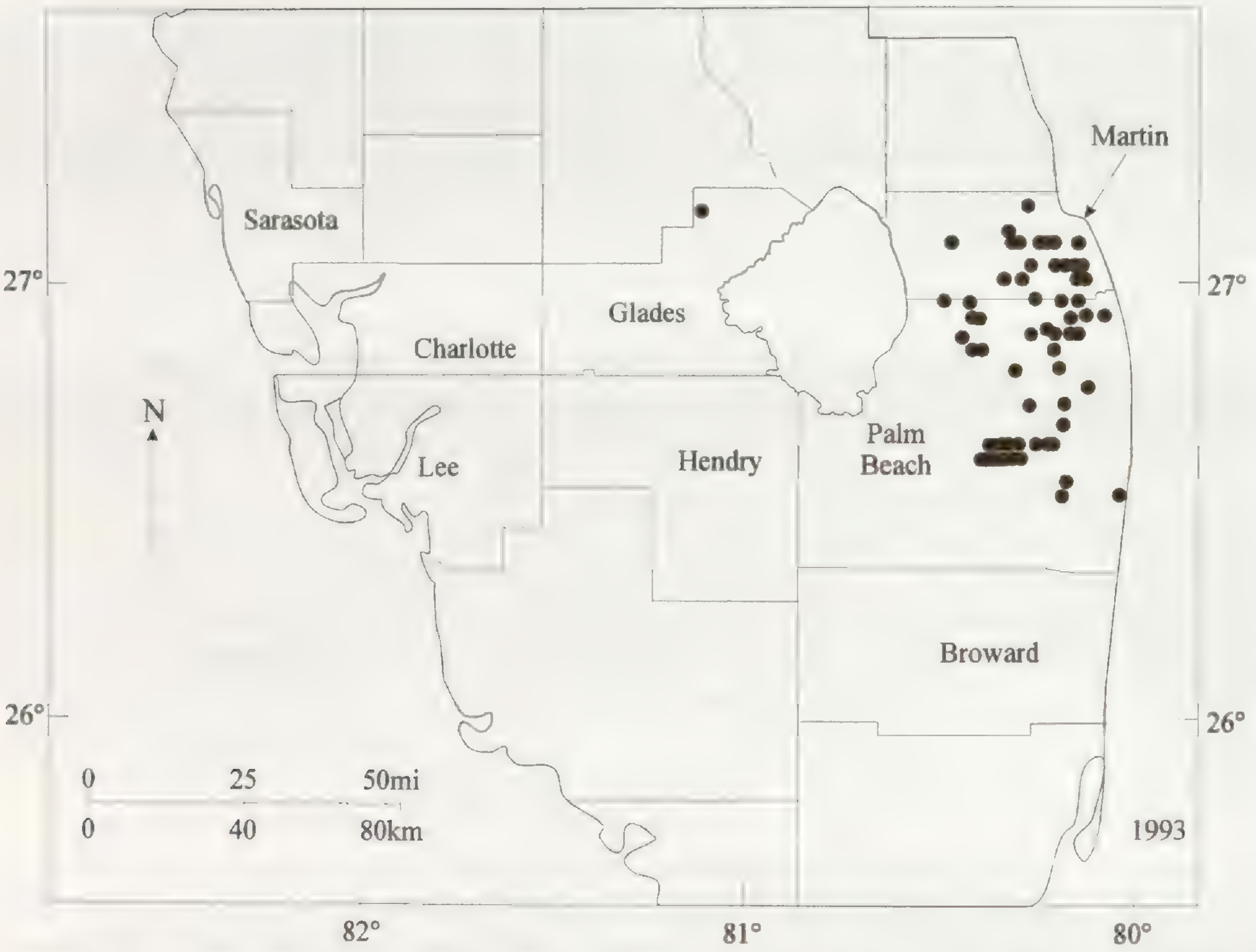

FIG. 4. Detected *Lygodium microphyllum* infestations in aerial surveys over southern Florida (from Lake Okeechobee south) in 1993.

data). Multiple naturalization events of this plant in different areas are also possible.

PROBABLE ORIGIN

Lygodium microphyllum has been cultivated as an ornamental plant for many years. It appears in the Royal Palm Nurseries (Manatee County, FL) 1888–89 catalogue. It was sold by this nursery for 30 years during 1888–1930 (Reasoner Brothers, 1887–1930). Royal Palm Nurseries, which operated from 1881 until the 1930's, was one of the most important importers and distributors of subtropical and tropical ornamental plants in Florida (and the United States) (Pinardi, 1980). It is possible that the plant they sold was, however, *L. japonicum* (Thunb. ex Murray) Sw. and not *L. microphyllum,* as the two were often confused in early horticultural literature. *Lygodium japonicum* is also a naturalized weed in Florida and the southeastern United States (Nauman, 1993), but to date is not as serious a problem as *L. microphyllum*. A photograph of *L. microphyllum* in the 1905 catalogue appears to be *L. japonicum*. Statements in these early catalogues indicate that the fern they sold is more hardy than other ferns (which "should be kept from hard frost"), which also suggests that the fern sold was *L. japonicum* rather than *L. microphyllum*. The

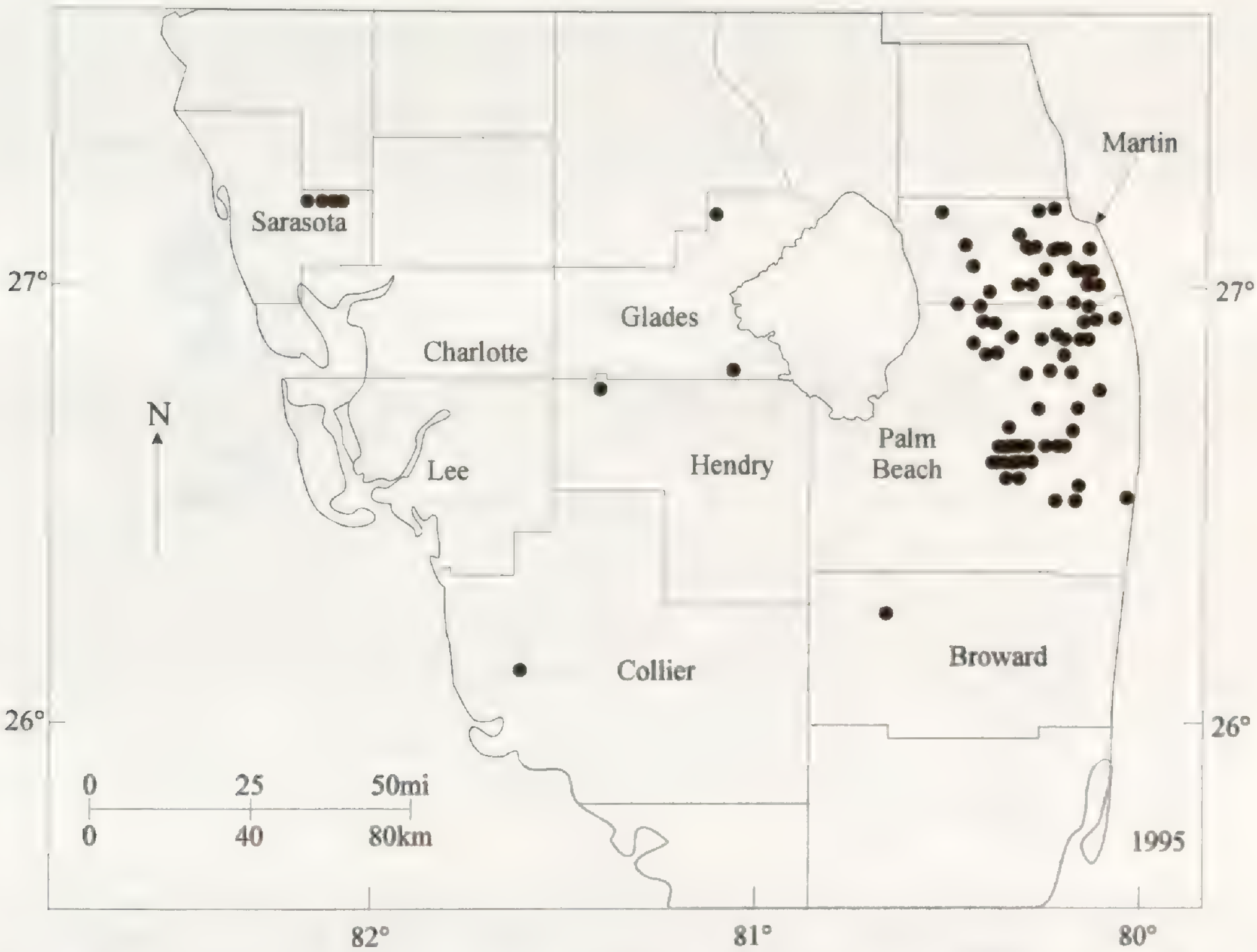

FIG. 5. Detected *Lygodium microphyllum* infestations in aerial surveys over southern Florida (from Lake Okeechobee south) in 1995.

native range of *L. microphyllum* is tropical and subtropical. Beckner (1968) thought that a clue to the fern's naturalization in Martin County was a collection from a nursery in Palm Beach county in 1958. The fern was also sold at a Polk County nursery in 1958 (Florida Division of Plant Industry, Gainesville, FL, Plant Disease Collection records), where it was found to be naturalized in 1979.

POTENTIAL TO SPREAD

Most of the natural areas in the southern third of Florida appear vulnerable to the invasion of *L. microphyllum*. The two major zones of the Everglades system, cypress swamps in the west and sawgrass with tree islands in the east, could be easily invaded and dominated by the fern. Pine flatwoods occupying large areas of the south central part of the state and many other areas are also susceptible to this fern. What the northern limit of *L. microphyllum* can be is unknown. The aboveground portion of the plant is killed by frost, but it can recover if the temperatures are not low enough to kill the roots. Roots growing in water, as they frequently do, experience additional protection from frost. The coolest areas *L. microphyllum* now occupies are interior sites in Polk, Hardee, and Highlands Counties (ca. 28°N). This suggests that the fern should

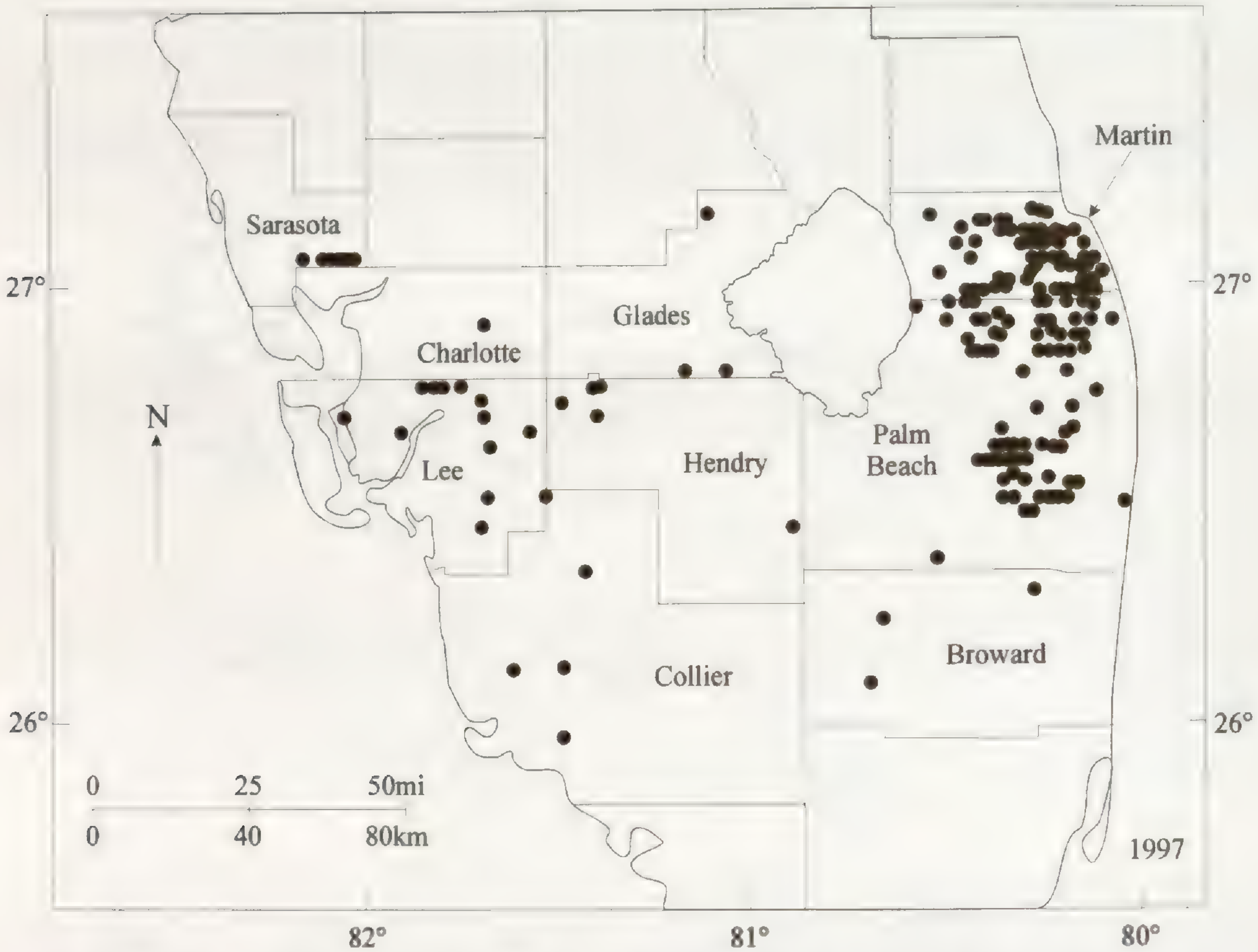

FIG. 6. Detected *Lygodium microphyllum* infestations in aerial surveys over southern Florida (from Lake Okeechobee south) in 1997.

be able to extend northward up both coasts (which are milder than the interior) to at least an equivalent winter low temperature zone that it occupies in Polk and Highland Counties. Polk and Highland Counties are in USDA Plant Hardiness Zone 9b which has average annual minimum temperatures of −3.9 to −6.6°C (Cathey, 1990). Zone 9b extends to Nassau County just south of the Georgia border on the east coast and Citrus County on the west coast. If the fern did reach Nassau County, it would be a 300 km and more than a 2° latitude extension of its current distribution. This would extend the plant farther into the temperate zone than it occurs in its native range. Florida appears to be warmer for its latitudes because of the Gulf of Mexico and the Gulfstream current flowing along the east coast. Mangroves (*Rhizhora* spp.) are usually limited to about 25° north or south latitude (Good, 1953), but reach 29° north latitude in Florida (Odum and McIvor, 1990).

It is possible for *L. microphyllum* spores to be carried by prevailing spring and summer winds from Florida across the Gulf of Mexico to Louisiana and Texas. The natural occurrence of this fern on some of the world's most remote islands, such as Mauritius and Tahiti (*Lorence 2227,* K; *MacDaniels 1284,* US), suggests an ability to disperse over very long distances. It may, however, take a long time for the fern to successfully colonize such remote places. The Gulf coast zone of southern Louisiana and Texas is in Cold Hardiness Zone 9b and

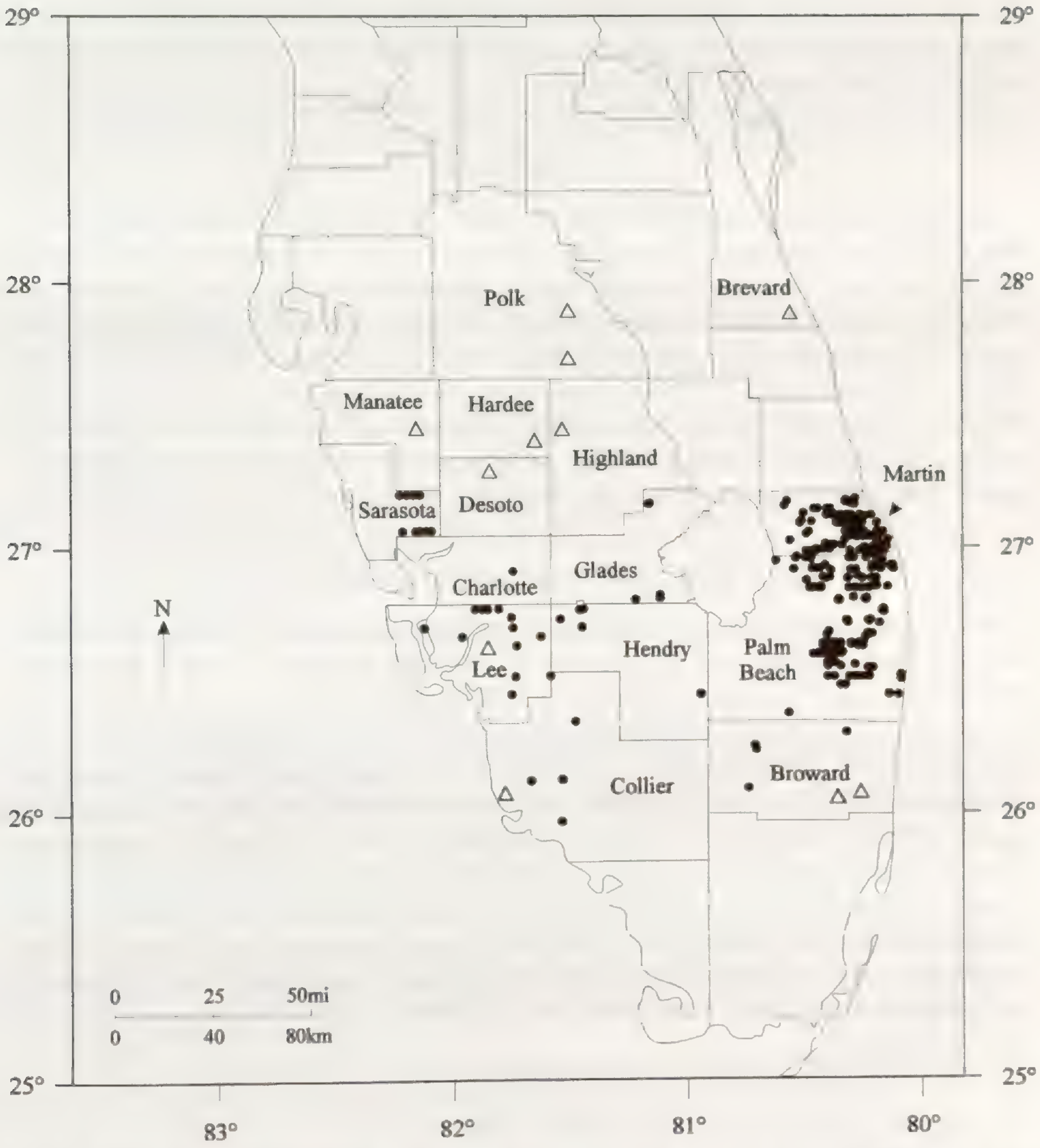

FIG. 7. Known total distribution of *Lygodium microphyllum* in 1997 in Florida. The black dots are aerial survey detection points and the triangles are herbarium specimen localities outside Martin and Palm Beach County.

the southern area of Texas is in zone 9a (Cathey, 1990). Mexico's Gulf coast is even warmer. The presence of abundant freshwater habitats in these areas, coupled with mild climates, suggests that much of the northwestern and western coast of the Gulf of Mexico are suitable for establishment, should *L. microphyllum* reach these areas. The West Indies and the Americas south of Florida seem less likely to be invaded because the prevailing winds are in the opposite direction (Henry et al., 1994). Spores might be carried southwest to Cuba by prevailing northeastern winds in the winter and perhaps by large

spiraling hurricanes. The populations of *L. microphyllum* in Jamiaca and Guyana could undergo local expansion and could be the source of spores that carry the fern to new areas in those regions.

Control

Limited research on possible control methods for this fern has been done to date. Work at Dickinson State Park indiciates that fire alone is not a good control method, but herbicide treatments, biomass removal, and follow-up herbicide applications show promise in controlling the fern (Roberts, unpublished report). The cost for this approach was about $9,000/acre, and there was significant damage to the native vegetation. Herbicide research is continuing (Stocker et al., 1997) but it will probably be difficult to control this vine with herbicides without damage to the valued native vegetation with which it grows. Biological control offers the most promise to control this species.

Other *Lygodium* Species in the United States

Two other *Lygodium* species occur in the United States. *Lygodium palmatum* (Bernh.) Sw. is a native to the eastern part of the country from Mississippi to New Hampshire (Nauman, 1993). It is distinguished from *L. microphyllum* by its deeply and palmately lobed sterile pinnae versus the unlobed usually oblong lanceolate shaped sterile pinnae in *L. microphyllum* (Fig. 1). *Lygodium japonicum* was found to be naturalized in Georgia at the turn of this century (Clute, 1903). This species occurs from eastern Texas through the southern states to North Carolina and Florida (Nauman, 1993). It is distinguished from *L. microphyllum* by its triangular sterile pinnae, which usually have an acute tip, proximate lobes, and serrate margins. *Lygodium japonicum,* native to temperate and tropical Asia (Ohwi, 1965; Singh and Panigrahi, 1984), can be invasive and is spreading, but it does not appear to be nearly as dangerous as *L. microphyllum*.

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The Potential of Biological Control to Manage Old World Climbing Fern (*Lygodium microphyllum*), an Invasive Weed in Florida

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ABSTRACT.—*Lygodium microphyllum* is native to wet tropical and subtropical Africa, Asia, Australia, and the Pacific islands. The genus *Lygodium* is taxonomically isolated and has only one native species in North America. Specialized natural enemies of *L. microphyllum* are predicted to exist that can be safely employed to control the fern in Florida, where it is a severe problem.

Lygodium microphyllum (Cav.) R. Br., or Old World climbing fern, is a serious weed in southern Florida, as described in the companion to this paper (Pemberton and Ferriter, 1998). In an attempt to reduce populations of this plant and to limit its spread, a biological control research effort is being initiated. The theory of biological control and the suitability of this approach to reduce and manage *L. microphyllum* are discussed. To help lay the groundwork for the biological control effort, the native range and taxonomic relations of the fern are defined. This information is used to plan and conduct surveys for natural enemies as well as research to define the potential host ranges of candidate biological control agents. The question of whether insects and other natural enemies can be expected to be associated with *L. microphyllum* is addressed.

TAXONOMY

The Old World climbing fern is usually called *L. microphyllum* but occasionally also *L. scandens* (L.) Sw. in some works. Although the name *L. scandens* was published earlier than *L. microphyllum* (1800 versus 1810), the name was based on other species of plants, not *L. microphyllum,* which means that *L. microphyllum* is the correct name and that *L. scandens* is an invalid name (Alston and Holttum, 1959). My examination of large numbers of specimens bearing both names from most of the native range and areas of introduction (material at K, BM, FLAS, GH, MICH, NY, US) suggests but one variable species.

Lygodium microphyllum is one of as many as 40 *Lygodium* species occurring in the tropical and warmer parts of the world (Mabberley, 1997). A current revision of the genus *Lygodium* reduces the number of species to 26 (J. Garrison, unpublished data). The genus *Lygodium* is usually placed in the Schizaeaceae, a small primitive family that also includes *Actinostachys, Schizaea, Anemia,* and *Mohria* (Prantl, 1881; Holttum, 1973). Because *Lygodium* is

unique morphologically, it is often interpreted to comprise its own monogeneric family, the Lygodiaceae (Bierhorst, 1971; Wagner and Smith, 1993). It is unclear what the relationships are between the genera that comprise the Schizaeaceae sensu lato (Smith, 1995). Recent *rbc*L work on the phylogeny of ferns found *Lygodium, Actinostachys,* and *Anemia* to have more intergeneric distance than occurs between most fern families (Hasebe et al., 1995). These genera were nevertheless called sister genera and left in the Schizaeaceae. The relationship of the Schizaeaceae to other groups of ferns is unclear (Smith, 1995). The *rbc*L analysis showed the family to be very isolated and to have more intrafamilial distance than occurs in other fern families (Hasebe et al., 1995). The *rbc*L data and the antiquity of the Schizaeaceae (*Anemia* spores are known from the Cretaceous [Smith, 1995]) suggest that the group diverged at an earlier time than most other groups.

Prantl (1881) separated the 22 species then recognized into three subgenera. These groups still appear to be valid (J. Garrison, pers. comm.). The subgenera are not separable by geography, and all three have species in the eastern and western hemispheres. The species are generally limited to a single continent—Americas, Asia, or Africa. *Lygodium microphyllum* is an exception, occurring in Africa, Asia, Australia, and the Pacific islands. It is the most widespread species in the genus. There are a few others that have very large ranges, such as *L. japonicum,* which occurs in both temperate and tropical Asia to Australia.

Lygodium palmatum (Bernh.) Sw.—the only *Lygodium* species native to the United States—is a temperate species recorded from Mississippi, Alabama, and Georgia northeast through New England (Nauman, 1993). It belongs to subgenus *Palmata.* Prantl's subgenus *Volubilia,* to which *L. microphyllum* belongs, contains seven species; one in tropical America (*L. volubile* Sw. in the West Indies including Cuba), three in tropical Asia; one in tropical Africa, one in Madagascar, and one in Polynesia. Other *Lygodium* species somewhat geographically near to *L. microphyllum* in Florida belong to the subgenus *Flexuosa* and include: *L. cubense* Kunth in Cuba; *L. venustum* Sw. in Mexico, the West Indies and south; *L. heterodoxum* Kunze in southern Mexico and Central America; and *L. oligostachyum* (Willd.) Desv., an endemic from Hispaniola (Dominican Republic and Haiti) (Judy Garrison, pers. comm.). The other climbing fern naturalized in the U.S., *L. japonicum,* also belongs to the subgenus *Flexuosa.*

Native Range

Lygodium microphyllum has an exceptionally large native range, occurring in much of the warm wet areas of the Old World (Fig. 1). The fern's temperate limits are between 28°S and 29°S in Australia and South Africa, and 25°N and 27°N in Assam (northeastern India) and the Ryuku Islands. It spans more than half of the world's circumference from 18°E in Senegal to 150°W in Tahiti. In Africa, *L. microphyllum* ranges from Senegal, south and east through most of West Africa, to Zaire, then south to Angola, east to East Africa, and south to

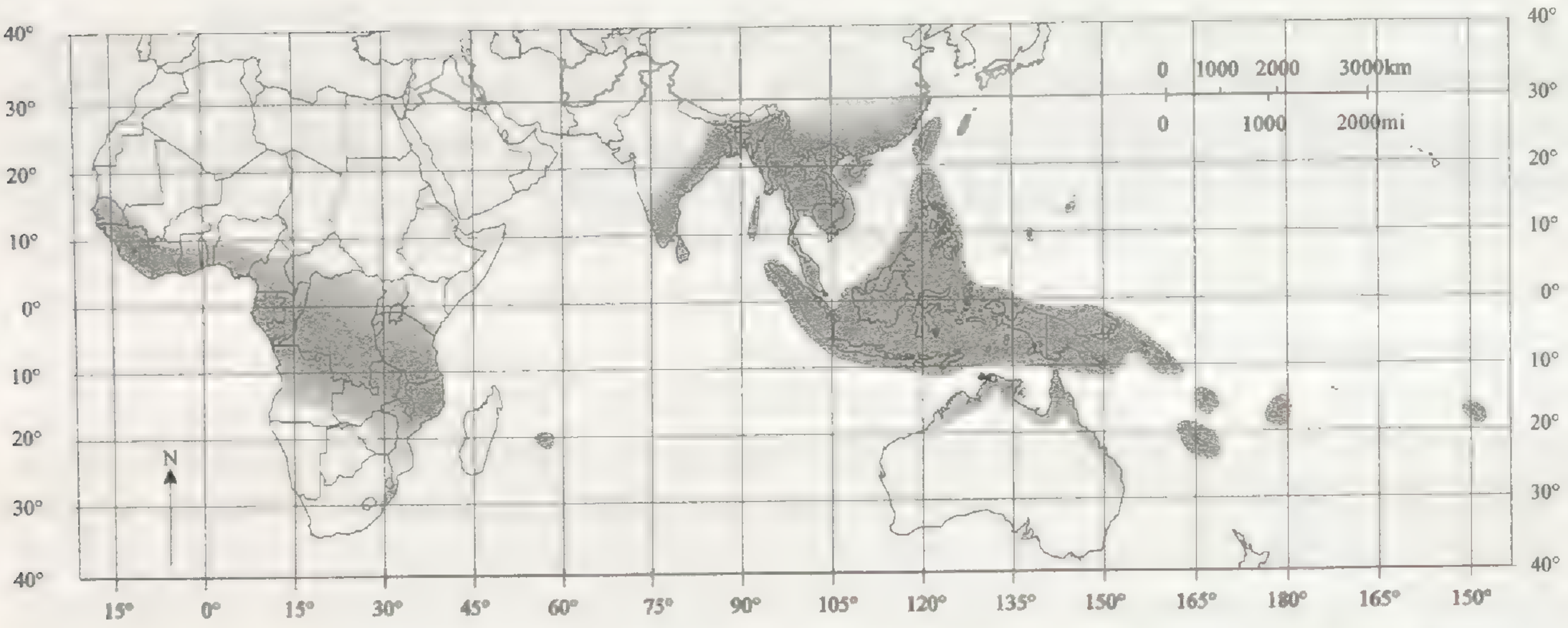

FIG. 1. Native distribution of Old World climbing fern (*Lygodium microphyllum*).

South Africa. It has been recorded in Senegal, Gambia, Guinea, Guinea Bissau, Mali, Sierra Leone, Liberia, Ivory Coast, Ghana, Benin, Nigeria (Alston, 1959), Central African Republic, Cameroon, Zaire, Angola, Uganda, Kenya, Tanzania, Zambia, Zimbabwe, Mozambique, South Africa (Schelpe, 1976), and Burundi (Lawalrée, 1970). In Asia, the fern is distributed from India, east through much of Southeast Asia, and north through the warmer provinces of southern China, to Taiwan and Okinawa. It also occurs throughout many of the Southeast Asian islands to Australia and the Pacific islands. It has been recorded from India and Sri Lanka (Singh and Panigrahi, 1984), China and Nepal, Indochina, Singapore, Indonesia, and New Guinea (Prantl, 1881), Thailand and Burma (Tagawa and Iwatsuki, 1979), Vietnam (Phram, 1970), Malaysia including Borneo (Holttum, 1968), Australia (Bentham, 1878; Clifford and Constantine, 1980), the Philippines (Copeland, 1958; So, 1994a), Hong Kong (Eddie, 1978; So, 1994b), Taiwan (Devol, 1975), Okinawa (Serizawa, 1975; Walker, 1976). In the Pacific, the fern occurs in the Marianas (Prantl, 1881), Solomon Islands and New Caledonia (Holttum, 1959), and Polynesia as far east as Tahiti (Holttum, 1968; *MacDaniels 1284,* US).

BIOLOGICAL CONTROL POTENTIAL

Biological control of a weed seeks insects and/or diseases of the plant in its native range and, after considerable host specificity research, introduces these against the plant in the area where it has become a problem. The plant's coevolved natural enemies are usually absent where the plant has been introduced and become a problem. The reassociation of these enemies with the problem plant can result in a reduction and regulation of the plant (Huffaker, 1964; Julien, 1992; Pemberton and Turner, 1980). Natural enemies of allied species (different *Lygodium* species) may also be employed to reduce the plant. The safety of biological control relates primarily to the selection of target pests that are introduced (non-native) and the use of biological control agents with very narrow host ranges (Pemberton, 1985a, b). If insects with host ranges that

include nontarget species or taxa are utilized, then effects on these nontarget species may occur. A project on exotic thistles carried out during the 1960's and 1970's resulted in control of the target thistles but also damage to nontarget native thistles (Louda et al., 1997). These negative effects were predictable and could have been avoided through either the use of agents with very narrow host ranges or through the selection of a pest target with fewer native relatives.

Most plants have very specialized insect predators (Strong et al. 1984) and to a lesser degree diseases that can be employed. Specialist insects typically have host ranges that are limited to plants in a genus, subgenus, section, or even single species. Specialist diseases can have similar host ranges, but some can be limited to subspecific taxa. Targeted weeds that are more distantly related to native plants or other valued plants can be expected to have both more natural enemies that can be used against it and fewer potential conflicts with valued plants. Biological control is a very suitable approach for *L. microphyllum,* because it is an introduced species that is taxonomically isolated from all but one U.S. native plant and a few Latin American species. Biological control is an appropriate tool for the control of this weed because it grows among other plants in complex natural vegetation that would be damaged by most control methods. Biological control has solved or lessened the severity of many exotic plants affecting natural areas (Dennill and Donnelly 1991, Pemberton and Turner 1990; Room et al., 1981). One of the most successful projects was against the floating aquatic fern *Salvinia molesta* D.S. Mitch. in Australia (Room et al., 1981).

Probability of Finding Natural Enemies

It was assumed that ferns were not generally eaten by insects (Soo Hoo and Fraenkel, 1964; Eastop, 1973; Southwood, 1973), but this was shown to be false (Balik et al., 1978; Gerson, 1979). Ferns in studied communities in Mexico experienced as much damage from insects as did angiosperms (Balik et al., 1978). These authors also searched the literature and found 420 named insect species associated with ferns. When the insect faunas of specific ferns have been studied, many specialist feeders of these ferns have been found. For instance, bracken fern (*Pteridium aquilinum* (L.) Kuhn sensu lato) in Europe has 33 insect species with narrow host plant ranges (Simmonds, 1967; Wieczorek, 1973; Lawton, 1976). Kirk (1977) discovered 24 insect species feeding on bracken in Papua New Guinea; most of them appeared to be specialists. Even the small, architecturally simple ferns in the genera *Salvinia* and *Azolla* have their own insects (Sands, 1983; Buckingham and Buckingham, 1981).

English-language literature searches have revealed almost no known insect feeders of *Lygodium* ferns. This is typical for tropical plants with little economic importance in their native regions. Most of the species associated with bracken in New Guinea were previously unknown (Kirk, 1977).

A similar lack of literature exists with plant pathogens of *Lygodium.* Some fungi have been collected from *L. japonicum* in Florida (Alfieri et al., 1994) and although little is known of these, they probably are not limiting the pest

Lygodium ferns. Of somewhat more interest is a rust fungus, *Puccinia lygodii* (Har.) Arthur. This rust is a pest of *Lygod*ium spp. in greenhouses (Jones, 1987). *Puccinia lygodii* is native to tropical South America (Sydow, 1925) and has been collected from *L. japonicum* in Louisiana (McCain et al., 1990). It is of interest because rusts often have very narrow host ranges and can be very damaging to their host(s). Although biological control usually uses natural enemies from the pest's native range, novel insects and diseases from closely related plants can be effectively employed as well (Julien, 1992).

Preliminary examinations of *L. microphyllum* at sites in its native range indicated the presence of insect feeders. I found pyralid moth larvae in Hong Kong; sawfly larvae (probably Hymenoptera, Tenthridinidae) were seen in Vietnam (M. Purcell, pers. comm.); and several moth larvae (Lepidoptera) were observed in Australia (C. Turner and K. Galway, pers. comm.).

These factors suggest that *L. microphyllum* should have a rich natural enemy assemblage in its native range. It will be important to find enemies that will not accept our native American *L. palmatum* as a host. Because *L. microphyllum* and *L. palmatum* are in different subgenera and live in different climatic zones, this should not be difficult. It will also be important to examine the potential use by prospective biological control agents of *Lygodium* species in the West Indies and Mexico. Representative ferns of many other taxa will also be included in host specificity testing.

Although biological control is a valuable tool for the management of introduced pest species, it does not always work. Sometimes a desired agent will not establish or develop high enough populations when introduced. Sometimes it is difficult to find natural enemies that are both host-specific and damaging to the pest plant. Biological control research is very good at predicting the potential host range (and therefore the safety of candidate agents), but it is difficult, if not impossible, to predict the population levels that an agent will have in the area that it is used. Populations of wild organisms are notoriously difficult to predict, primarily because we cannot predict weather, and therefore weather's important direct and indirect influences on population change. Because almost half of the attempted biological control of weeds projects have resulted in population reduction of the target pest (Office of Technology Assessment, 1995), it is worthwhile to attempt, especially against such an invasive plant as *L. microphyllum*.

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American Fern Journal 88(4):183 (1998)

Shorter Note

A New Combination in *Colysis*.—The genus *Colysis* (Polypodiaceae) was first described by Presl (Epimel. bot. 146, 1851). Dixit (*A census of the Indian pteridophytes,* Botanical Survey of India, Howrah, 1984) enumerated about eight species for India, but Manickam and Irudayaraj (*Pteridophyte flora of the western Ghats—south India,* B. I. Publications, New Delhi, 1992) included only one species for southern India, *C. hemionitidea* (Wall. ex Mett.) C. Presl. Recently, in studying some putative interspecific hybrids among Indian *Colysis,* Manickam et al. (Taxon 46:265–268, 1997) added one more species to the genus by transferring *C. decurrens* (Blume) Manickam & Irudayaraj from the genus *Leptochilus.* This was done in agreement with the views of Hetterscheid and Hennipman (Bot. Jahrb. Syst. 105:11–47, 1984), who adopted a broad circumscription of *Colysis* to include a series of former segregates including *Leptochilus,* based on patterns of variation in frond shape and venation, as well as characteristics of the sori.

Manickam and Irudayaraj (1992) also treated *Leptochilus thwaitesianus* Fée as a distinct species, which has not been transferred to the genus *Colysis.* This taxon also was accepted as a distinct species by Sledge (Bull. British Mus. Nat. Hist. Bot. 2(5):14, 1960) in his research on the genus in Ceylon. The following new combination is presented here:

Colysis thwaitesianus Rajkumar & Arunachalam comb. nov.—Basionym: *Leptochilus thwaitesianus* Fée, Mém. foug. 10:7, pl. 24. 1865.

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Referees for 1998

Maintaining the quality of papers published in the American Fern Journal is perhaps one of the most difficult tasks the editor faces. Submissions cover a diversity of topics. The willingness of the following referees to undertake detailed reviews of the often challenging manuscripts helped to maintain the journal's high standards of scientific and journalistic excellence. The American Fern Society is indebted to these individuals for their service, as is the editor.—
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